A SAFETY AND EFFICACY STUDY OF ADDING LOW DOSE PEGYLATED IFN-ALPHA 2B TO STANDARD DOSE DASATINIB IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA

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**Study Sponsor:** Norwegian University of Science and Technology (NTNU) on the behalf of Nordic CML study group (NCMLSG) Høyskoleringen 1, N-7491 Trondheim Norway, through the Dept of Cancer Research and Molecular Medicine (IKM). (Chief of Dept Prof Magne Børset).

**Main Contact Name:** Principal Investigator: Henrik Hjorth-Hansen, Trondheim, Norway; Study Coordinator: Satu Mustjoki, Helsinki, Finland

**Study Management Committee:** Henrik Hjorth-Hansen, Trondheim, NO; Satu Mustjoki, Helsinki, FI; Jesper Stentoft, Aarhus, DK; Ulla Olsson Strømberg, Uppsala SE; Johan Richter, Lund, SE; Leif Stenke, Stockholm, SE; Ole Weis Bjerrum, Copenhagen, DK; Kimmo Porkka, Helsinki, FI; Franz Gruber, Tromsø, NO

**Investigators:** Henrik Hjorth-Hansen (PI) (Trondheim).

Denmark (DK): Jesper Stentoft (National Coordinator, NC) (Aarhus), Ole Weis Bjerrum (Copenhagen), Finland (FI): Satu Mustjoki (NC), Kimmo Porkka, Perttu Koskenvesa, (all Helsinki)

Norway (NO): Tobias Gedde-Dahl (Oslo), Bjørn-Tore Gjertsen (Bergen), Waleed Majeed (Stavanger), Franz Gruber (Tromsø), and Henrik Hjorth-Hansen (Trondheim)(NC).

Sweden (SE): Ulla Olsson Strömberg (Uppsala)(NC), Johan Richter (Lund), Leif Stenke, Lotta Ohm and Sören Lehmann (Stockholm), Berit Markevärn (Umeå), Mats Björeman (Örebro), Claes Malm (Linköping), Anders Själander (Sundsvall), Kristina Myhr Eriksson (Luleå)

**Molecular Monitoring Committee:** Hans Ehrencrona (Lund, leader), Veli Kairisto (Turku), Aleksandra Silye (Oslo) Charlotte Nyvold (Aarhus).

**Safety Monitoring Committee:** Professors Johan Lanng Nielsen, Aarhus, Finn Wisløff,Oslo and Ingemar Turesson, Malmö.

**Mailing Address:** Henrik Hjorth-Hansen: Dept of Hematology, St. Olavs Hospital, NO-7006 Trondheim, Norway

**Phone/Fax:** Henrik Hjorth-Hansen: Tel: +47 72825100 Mob +47 97121240 Fax +47 72571465  
Satu Mustjoki: Tel +358 947171898 Mob+358 405521606 Fax +358 947171890

**E-mail Address:** henrik.hjorth-hansen@ntnu.no satu.mustjoki@helsinki.fi

**Products:** Dasatinib (Sprycel, Bristol-Myers Squibb) Pegylated interferon-α2b (PegIntron, Merck)

**Indication:** Patients with newly diagnosed CML in chronic phase

**Phase:** Ib/IIta

**Study Design:** Open-label, single arm
**Study rationale and research hypothesis:** Patients with newly diagnosed CML have excellent outcomes with tyrosine kinase inhibitors (TKI). However, a few patients will be cured with TKIs alone, and thus need continued life-long treatment. Some patients achieve complete molecular remission (CMR), and this rate is higher with second generation TKIs compared with imatinib.\(^1\,^2\) Some experience with drug discontinuation in CMR has been derived from a few small studies, most notably the French STIM study.\(^3\) Approximately 40% of patients with a minimum of two years in MR4.5 (4.5 log reduction in molecular response) can stop imatinib without relapse, indicating possible cure. To increase the non-relapse rate is of major importance. To achieve a permanent “cure” without stem cell transplantation is presently the most relevant goal of clinical studies in CML.

We hypothesize that to significantly increase cure rates in CML, therapy should eradicate leukemic stem cells and/or induce or restore anti-CML immunity. Second generation TKIs may have a more profound effect on the stem cell pool as compared to imatinib. This is assessed in our current randomized study with a reduction in leukemic stem cell burden as the primary endpoint (NordCML006). Interferon-alpha (IFN) has a prominent immunomodulatory and antiproliferative mode of action, and has also activity in stem cells. Pegylated IFN in combination with imatinib results in improved therapy responses as compared to imatinib monotherapy.\(^4\,^5\) This advantage may translate into higher cure rates.

Dasatinib has a unique dual mechanism of action: it is the most potent of available TKIs and induces immunological effects that are different from those of IFN. Both of these drugs may have immunological adverse-effects when used as a monotherapy. However, immunological adverse-effects may also be markers of anti-leukemia efficacy. A combination of dasatinib and pegylated IFN (PegIFN) may have additive or synergistic effects and should be tested in a clinical study.

In addition, it would be important to understand mechanisms of drug-induced cure. A very important element of this protocol is the biomarker program, which aims to identify markers relevant to disease biology and therapy outcome.

**Rationale for selected dose and schedule outlined for all treatment arms:**

**The study design** consists of a debulking and stabilization phase of three months (Dasatinib 100mg once daily [OD]), a combination phase of 12 months (Dasatinib+ PegIFN), and a consolidation phase of 9 months (Dasatinib 100 mg OD). Dasatinib is EMEA-approved for first line treatment of CML-CP and can be considered standard of care.
After three months a low dose of PegIFN (PegIntron, Merck) (15 μg subcutaneous injection once weekly for 12 months) is added. If not hampered by unacceptable toxicity in the first 15 patients, a total of 35 patients will be included. The dose and duration of PegIFN is based on our experience from the NordCML002 study, where imatinib was combined with PegIFN at a mean dose of only 24μg/week (many discontinuations). In spite of this, the combination was considerably more efficacious than imatinib alone. If a patient tolerates 15μg weekly well after 3 months on the combination, an increase to 25μg/week is scheduled.

**Treatment:** Dasatinib 100mg OD for three months single drug, with subsequent addition of PegIFN 15 μg/week for 3 months. If well tolerated (less than grade 2 non-hematological or grade 3 hematological AE) the dose should be increased to 25μg weekly for the remaining 9 months on combination treatment. Thereafter dasatinib will be given as monotherapy. Patients will be followed for 24 months totally.

**Primary Objective:** To study the efficacy and safety of combination treatment in newly diagnosed CML patients in chronic phase. A later phase III randomized study may be warranted if MMR (major molecular response, defined as ≤0.1% BCR-ABL1 on the International Scale) rates after 12 months are better than in the DASISION study (>46%) and if toxicity of the combination is not prohibitive.

**Primary endpoint:**

**Safety endpoint in run-in phase (first 15 patients):** Study stops if four out of the first five, six of the first 10 or 8 of the first 15 patients experience grade IV hematological toxicity, grade III non-hematological toxicity or grade II serosal effusions during first 6 months of treatment. Data will be evaluated by an independent safety committee.

**Whole study efficacy endpoint:** Rate of MMR after 12 months.

**Secondary Objective(s):** Efficacy and toxicity also compared to the DASISION registration study of dasatinib in 1st line treatment and to the NordCML006 study (NCMLSG study, dasatinib 100mg vs. imatinib 400mg monotherapy). Adherence to PegIFN. Disease progression. Quality of life. Effect of tumor burden in stem cells on treatment outcome. To perform immunological and other lab studies to explain effects and toxicity, and to attempt to identify biomarkers for response.

**Secondary Endpoint (s)**

- Rate of CCgR after 3, 6, 12 and 18 months
- Rate of MMR after 3, 6, 15, 18, and 24 months
- Rate of MR4.0 and MR4.5 after 3, 6, 12, 15, 18, and 24 months
Rate of grade 2 non-hematological toxicity that poses a clinical problem (duration more than one month in spite of symptomatic treatment)

Rate of grade 3-4 hematological toxicity

Overall survival

Rate of patients who complete 3, 6 and 12 months of PegIFN treatment

QoL at 0, 3, 6, 12 and 18 months

Progression to advanced disease phase.

Comparison of safety and efficacy variables with historical cohorts from Nord CML006 and NordCML002

**Biomarkers** of response, failure and toxicity.  

**A)** Fraction of leukemic cells in the stem cell (Ph+ CD34+CD38- cells) and progenitor cell (Ph+ CD34+CD38+) compartment at debut and 4 weeks of dasatinib treatment.  

**B)** Lymphocyte subpopulations at 0, 3 and 6 months by flow cytometry assay: Enumeration of CD4+ and CD8+ T-cells, NK-cells, NKT-cells, B-cells and regulatory T-cells. Correlation of these subpopulations to response.  

**C)** Functional assays of lymphocytes: Cytotoxicity of NK-cells. IFN-γ production by T cells after stimulation. Granzyme B staining for evaluating the cytotoxic potential of T-cells. Functional assays will be performed both from pre- and post-drug samples.  

**D)** Clonality: TCR γδ rearrangements by PCR  

**E)** Plasma cytokine array: Luminex multiplex system  

**F)** Proteomics

A simplified follow-up of treatment response by real-time quantitative reverse transcriptase PCR (RQ-PCR), treatment type, progression to advanced phase and survival will be performed yearly until end of year 5.

**Sample Size Justification:** Concerning toxicity: In the French Spirit study the rate of grade 3-4 *non hematological toxicity* over 24 months was 35% in the combination arm and 10% in the imatinib arm. In the DASISION study there were 3-4% grade 3 events in both arms after 12 months. Grade 3-4 *hematological toxicity* in the Spirit study was 63% in the combination arm and 12% with Imatinib. In the DASISION study the numbers were 37% on Imatinib and 50% on dasatinib. Increased number and severity of side effects are expected with the combination of dasatinib and PegIFN, with hope of increased efficacy. A 50% rate of grade 4 hematological or grade 3-4 non-hematological toxicity is perceived as a limit for what patients and treating physicians will accept in pursuit of deeper and faster responses and possible future cure. This must be viewed in light of the excellent efficacy, simplicity and tolerability of single drug TKI treatment. An increase of pleural effusion from the estimated 10-20% on
dasatinib alone (DASISION, and our own experience in the NordCML006 protocol) or < 2% on IFN+ Imatinib, to 50% is also judged to indicate non-feasibility. Concerning efficacy: Using the Fleming one-stage approach expecting a clinically meaningful difference to be minimally 23% increase in MMR rate (from 46% in DASISION to 69% in this study) to be clinically interesting. By strict analysis, 30 patients are needed for the efficacy estimate using Fleming’s method, with dismissal of the null hypothesis if > 19 patients achieve MMR. To avoid statistical power problems we plan inclusion of 35 patients for a reasonable estimate of efficacy and safety.

1. **Inclusion Criteria:**
   2. Age ≥18-70 years of age
   3. Diagnosis of chronic myeloid leukemia in chronic phase (CML-CP) associated with BCR-ABL1 quantifiable by RQ-PCR (IS)
   4. No other current or planned anti-leukemia therapies excluding hydroxyurea treatment for up to two months.
   5. ECOG Performance status 0,1, or 2
   6. Adequate organ function as defined by: Total bilirubin < 1.5 x ULN (ULN = upper limit of normal in a local institution lab) in absence of Gilbert genotype; ASAT and ALAT < 2.5 x ULN. Creatinine < 2x ULN. Potassium, magnesium and phosphate not below LLN (LLN= lower level of normal)
   7. Life expectancy of more than 12 months in the absence of any intervention
   8. Patient has given written informed consent to participate in the study

**Exclusion criteria:**
1. Prior accelerated phase or blast crisis
2. Uncontrolled or significant cardiovascular disease, including any of the following:
   - A myocardial infarction within 6 months
   - Uncontrolled angina within 3 months
   - Congestive heart failure within 3 months
   - Diagnosed or suspected congenital long QT syndrome
   - Any history of clinically significant ventricular arrhythmias (such as ventricular tachycardia, ventricular fibrillation, or Torsades de Pointe)
   - Prolonged QTcF interval > 450 msec on pre-entry ECG
3. Atypical BCR-ABL1 transcript not quantifiable by RQ-PCR.
4. Another primary malignant disease, which requires systemic treatment (chemotherapy or radiation)
5. Severe and/or life-threatening medical disease including acute liver disease
6. History of significant congenital or acquired bleeding disorder unrelated to cancer
7. Impairment of gastrointestinal (GI) function or GI disease that may significantly alter the absorption of dasatinib
8. Patients actively receiving therapy with strong CYP3A4 inhibitors and the treatment cannot be either discontinued or switched to a different medication prior to starting study drug
9. Patients who are currently receiving treatment with any medications that have the potential to prolong the QT interval and the treatment cannot be either discontinued or switched to a different medication prior to starting study drug
10. Female patients who are: pregnant, breast feeding or potentially fertile without a negative pregnancy test prior to baseline or unwilling to use contraception on trial
11. Previous history of pericarditis or pleuritis
12. History of non-compliance, abuse of alcohol, illicit drugs, severe psychiatric disorders or other inability to grant informed consent.
14. Hypersensitivity to any interferon preparation;
15. Autoimmune hepatitis or a history of autoimmune disease;
16. Pre-existing thyroid disease unless it can be controlled with conventional treatment;
17. Epilepsy and/or compromised central nervous system (CNS) function;
18. HCV/HIV patients

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**Statistics**

For **toxicity**: Frequencies of different grades and types of AE will be calculated and tabulated with similar data from NordCML002, NordCML006 and DASISION

For **efficacy**: To compare with historical cohorts (mainly the registration study DASISION, but also NordCML002 and 006) we base the following assumptions. With 35 patients one can detect a 23% difference in MMR rates with a statistical power of 80%. This means 69% MMR compared to historical 46% in DASISION. The absolute increase in MMR rate with pegylated interferon found in French SPIRIT and NordCML002 (20% and 28%) is in this range. Using the Fleming one-stage approach expecting a clinically meaningful difference to be minimally a 23% increase in MMR rate (from 46% in DASISION to 69% in this study). By strict analysis, 30 patients are needed for the efficacy estimate using Fleming’s
method, with dismissal of the null hypothesis if 19 or more patients achieve MMR. Using 35 patients, the dismissal of the null hypothesis occurs at 23 or more patients achieving MMR.

**Population:**
35 patients with newly diagnosed CML in chronic phase (CP)

**Study Duration:**
24 months

**Study Milestone Dates:**
Study start (FPFV): Sept 2012
Recruitment end (LPFV): Q4 2013.
Study end (LPLV): Q4 2015

Publication date: Preliminary publication of safety and efficacy data after interim safety analysis is possible early 2014. Mature data: December 2015 (ASH) or May 2016 (EHA) and manuscript submitted to peer-reviewed journal

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**TABLE OF CONTENTS**

Introduction ............................................................................................................................................................................................. 10
Overview of chronic myeloid leukemia ............................................................................................................................................... 10
Short overview of pathophysiology ................................................................................................................................................ 10
Imatinib treatment in CML chronic phase (CP) ............................................................................................................................... 10
CMR induced by TKIs and potential for “cure” ............................................................................................................................... 10
Overview of dasatinib (Sprycel) ...................................................................................................................................................... 11
Overview of IFN-alpha 2b in CML ................................................................................................................................................... 14
Study rationale and purpose ............................................................................................................................................................... 16
Objectives and endpoints of the study ................................................................................................................................................ 17
Primary objectives: .......................................................................................................................................................................... 17
Secondary objectives: ..................................................................................................................................................................... 18
Primary endpoint: ........................................................................................................................................................................... 18
Secondary endpoints: ...................................................................................................................................................................... 18
Study design ........................................................................................................................................................................................ 19
Inclusion Criteria: ..................................................................................................................................................................... 19
Exclusion criteria: ........................................................................................................................................................................... 20

Informed Consent procedures............................................................................................................................................................. 21
Update of Informed Consent .......................................................................................................................................................... 21

Treatment............................................................................................................................................................................................ 21
Investigational and control drugs.................................................................................................................................................... 21
Patient numbering ............................................................................................................................................................................ 22
Patient treatment............................................................................................................................................................................. 22
Adjustment of study drug dose (general principles)....................................................................................................................... 24

Concomitant medications and interactions .................................................................................................................................... 28
Study drug discontinuation ............................................................................................................................................................. 31
End of study .................................................................................................................................................................................... 31
Discontinuation from study............................................................................................................................................................. 32

DEFINITIONS OF RISK, DISEASE PHASE AND RESPONSE ............................................................................................................ 32

Visit schedule and assessments........................................................................................................................................................ 35
Screening Activities ......................................................................................................................................................................... 35
Required Procedures ....................................................................................................................................................................... 35
Information to be collected on screening failures .......................................................................................................................... 39
Patient demographics/other baseline characteristics .................................................................................................................... 39
Safety Assessments ............................................................................................................................................................................ 41
Pregnancy ........................................................................................................................................................................................ 43
Safety monitoring committee (SMC) and interim analysis. ............................................................................................................ 44
Data review and data management .................................................................................................................................................... 44
Data collection, database management and quality control: ......................................................................................................... 44
Statistical methods and data analysis .................................................................................................................................................. 44
Populations for analysis .................................................................................................................................................................. 44
Primary endpoint ................................................................................................................................................................................. 45
Secondary objectives and exploratory analysis ............................................................................................................................ 45
Ethical issues and administrative procedures ..................................................................................................................................... 47
Ethical considerations ........................................................................................................................................................................ 47
INTRODUCTION

OVERVIEW OF CHRONIC MYELOID LEUKEMIA

SHORT OVERVIEW OF PATHOPHYSIOLOGY

Chronic myeloid leukemia (CML) is a hematological stem cell disorder associated with a specific chromosomal translocation known as the Philadelphia (Ph) chromosome detected in 95% of patients.6;7 The molecular consequence of the translocation is the fusion of the Abl proto-oncogene to the Bcr gene resulting in the production of an activated form of the Abl protein-tyrosine kinase.8;9 Expression of the BCR-ABL1 protein is capable of inducing leukemias in mice, implicating the protein as the cause of these diseases.10;11

Clinically, CML progresses through three distinct phases of increasing refractoriness to therapy: chronic phase (median duration 3-4 years prior to the imatinib era, accelerated phase (median duration 3-9 months; median survival 8-18 months), and blast crisis (median survival 3-6 months). Most patients are however diagnosed in the chronic phase, characterized by splenomegaly and leukocytosis with generally few symptoms.

IMATINIB TREATMENT IN CML CHRONIC PHASE (CP)

Treatment of CML-CP has been greatly improved since the introduction of TKI inhibitors. Imatinib induces rapid hematologic, cytogenetic and molecular responses. In a long-term follow-up of the IRIS study, the cumulative rate of complete cytogenetic response (CCyR) to imatinib was 69% at 12 months and 87% at 60 months.12

The degree of BCR-ABL1 reduction is clinically relevant as a higher rate of BCR-ABL1 reduction in the IRIS study was associated with improved PFS.13 In a subset of IRIS patients treated in Australia and consistently monitored for molecular response by RQ-PCR (n=53,) a continuous decline in BCR-ABL1 was observed in a 7-year follow-up. The BCR-ABL1 reduction was not linear. The median time to a 3-log reduction (same as MMR) was 18 months, time to a 4-log reduction was 45 months and time to undetectable BCR-ABL1 (> 4.5 reduction) was 66 months.14 The relapse rate after achievement of MMR is very low, and is regarded as a “safe haven” and a goal of treatment.13

CMR INDUCED BY TKIS AND POTENTIAL FOR “CURE”

Inspired by the French stopping project, there is now more and more focus on the possibility to cure patients with CML, and an increasing focus on CMR as a treatment goal on the way to “cure”.3 In Mahon’s study approximately 40 % of patients in CMR4.5 did not relapse after stop of therapy. Shorter duration of imatinib treatment than 50 months, and high risk score according to Sokal identified patients with smaller chances of remaining relapse-free.

Both 2nd generation TKIs, Dasatinib and Nilotinib induce more frequently CMR than imatinib in the upfront situation, indicating a superior outcome with the use of 2nd generation TKIs as compared to
imatinib. In addition, combination of imatinib with PegIFN induces more CMR than imatinib alone. It is logical that the combination of a 2nd generation TKI and PegIFN is likely to be more efficacious than TKI alone.

OVERVIEW OF DASATINIB (SPRYCEL)

Dasatinib was developed as an inhibitor of src family kinases and is known to inhibit a large number of tyrosine kinases. Since the BCR-ABL1 tyrosine kinase in its active conformation is structurally very similar to src kinases, dasatinib was tried in cells and patients with mutations conferring resistance to imatinib. Dasatinib is more than 300 times as potent as imatinib with activity in the low nanomolar range. Dasatinib showed remarkable efficacy upon oral administration, and was subsequently used for imatinib-intolerant and resistant patients (2nd line) and eventually in 1st line. Dasatinib is rapidly absorbed following oral administration in subjects with Cmax values attained at median 1 h (t½ was 3-5 hs). Dasatinib has an apparent volume of distribution of 2505 liters, suggesting that the drug is extensively distributed in the extravascular space. There is no significant influence on pharmacokinetic parameters of food intake. Dasatinib is metabolized mostly through CYP3A4 with mainly fecal elimination of metabolites. Only 4% of radiolabelled dasatinib were eliminated through the urinary tract.

2nd LINE EXPERIENCE:

Dasatinib is effective in patients with CP CML. This is based primarily on the results of a large Phase II study (CA180013), in which 387 CP CML patients resistant or intolerant of imatinib were treated with dasatinib. In imatinib-resistant patients, the MCyR rate was 42% and the CCyR rate was 30%. The results of this single-arm Phase II trial were confirmed in study CA180017, a randomized Phase II study of dasatinib 70 mg BID vs. imatinib 800 mg in chronic CML patients resistant to ≤ 600 mg imatinib. In this trial, 150 patients were treated, randomized 2:1. Improved responses (CCgR 35% vs 16%) and remarkably fewer treatment failures were noted, 23% vs 80% for dasatinib-treated vs imatinib-treated patients. The randomized phase 3 study CA180034, demonstrated the superior efficacy and safety profile of dasatinib 100mg OD.

1st LINE EXPERIENCE:

A single arm phase 2 study using dasatinib 100 mg OD in newly-diagnosed chronic phase CML shows a 98% CCgR rate and 81% MMR rate after 12 months of treatment. Based on these trials a randomized phase 3 trial of dasatinib 100mg OD vs imatinib 400 mg OD was performed, the so called DASISION trial. Responses were significantly faster and deeper with dasatinib than with imatinib i.e after 12 months 83% vs 72% achieved a CCyR, 46% vs 28% achieved MMR and a later presentation demonstrated improved rate of MR 4.5 from 13% vs 7%, all statistically significant. There were fewer progressions to accelerated or blast phase on treatment, 1.9 vs 3.5%, but no survival benefit 97% vs 99% respectively. Based on the DASISION study, dasatinib has been approved 1st line by EMEA and FDA, and is reimbursed
by some insurance organizations in the Nordic countries on this indication. Our own phase 2 study of dasatinib vs imatinib frontline, also shows superior efficacy of dasatinib 12 months post randomization (88% vs 40% MMR, 44% vs 7% MR4.0) (Mustjoki, ASH 2011, oral presentation).

**DASATINIB SAFETY**

Very detailed records of adverse events AEs are available in the SmPC (see also FASS, Felleskatalogen, DK and FI). Toxicity data (see facsimiled Table 4) from the DASISION study give a good record on the most frequent and serious side effects of dasatinib. Hematological side effects are frequent with dasatinib administration, with similar rate of neutropenia, and more grade 3 and 4 thrombocytopenia (19% vs 10%) than imatinib. Patients entering this study will be treated with dasatinib for 3 months before combination with PegIFN and will mostly be in stable CHR without cytopenias. Of non-hematological toxicity, pleural effusion and rarely pericarditis are distinct dasatinib-related side effects. The occurrence of serositis is associated with good responses, release of clonal large granular lymphocytes in peripheral blood, and an anti-leukemic effect has been implicated. Patients with serosal effusions should interrupt dasatinib, and about one fourth will get recurrence of effusion and need to discontinue the drug. Serosal effusion is always reversible and often steroid sensitive. Many imatinib-treated patients have mild abdominal discomfort, eyelid edema, rash, cramps and musculoskeletal pain all of which are much less frequent with dasatinib. Recently the occurrence of pulmonary arterial hypertension (PAH) has been reported with dasatinib, and Bristol-Myers Squibb has issued a letter to physicians in this regard. PAH is rare, but should be considered as a differential diagnosis in case of thoracic and pulmonary symptoms. If not a commoner cause of dyspnea, such as pleural effusion is found, a work-up for PAH is indicated. See below in the “dose modifications for PAH” section p 29

**SAFETY IN THE DASISION STUDY**

Most patients (>98%) experienced at least one AE. The most frequently affected system organ classes (SOCs) were skin and subcutaneous tissue disorders and gastrointestinal disorders. A summary of most frequently reported study drug-related AEs 12 months (in ≥ 10% of patients in any of the treatment groups) is presented in facsimiled Table 4.
Adverse events (AEs) were generally managed either with a dose reduction, dose interruption, or supportive care. The AEs seen in the study were overall as expected for this population, class of drugs and is consistent with the known safety profiles dasatinib and imatinib.

Table 4. Drug-Related Adverse Events That Occurred in at Least 10% of Treated Patients.

<table>
<thead>
<tr>
<th>Event</th>
<th>Dasatinib (N = 258)</th>
<th>Imatinib (N = 258)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Grades</td>
<td>Grade 3 or 4</td>
</tr>
<tr>
<td></td>
<td>% of patients</td>
<td></td>
</tr>
<tr>
<td>Cytopenia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutropenia</td>
<td>65</td>
<td>21</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>70</td>
<td>19</td>
</tr>
<tr>
<td>Anemia</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>Nonhematologic adverse event</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluid retention</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>Superficial edema</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>17</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Nausea</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Myalgia</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Muscle inflammation</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Musculoskeletal pain</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Rash</td>
<td>11</td>
<td>0</td>
</tr>
</tbody>
</table>
CARDIOTOXICITY AND SUDDEN DEATHS

In all studies with TKIs, patients with overt cardiac disease including arrhythmias have been excluded, probably to avoid setbacks in drug development. Cases of sudden death have been reported, but it is difficult to assess them as drug-related or not. Nine of the 1150 dasatinib-treated patients with chronic phase CML had QTc prolongation reported as an adverse event. Of these 7 were considered related to drug. None of the nine patients who reported QTc prolongation used 100mg QD, eight used 70 mg BID. Although the authors of this protocol believe that the documentation in regard to cardiac disease clearly speaks against serious toxicity, we have decided to exclude patients with cardiac problems from this trial (see exclusion criteria).

OVERVIEW OF IFN-ALPHA 2B IN CML

Before the introduction of imatinib, interferon-α (IFN-α)-based regimens were preferred for upfront treatment of patients not eligible for allogeneic stem cell transplantation.21 In vitro, IFN-α has a synergistic effect in combination with imatinib.22 IFN-α is, however, barely detectable in the serum 24 h after its administration, thus warranting frequent administration (daily to 2-3 times weekly) for sustained efficacy. To overcome this limitation, two forms of pegylated (covalent attachment of polyethylene glycol, Peg) IFN-α have been developed (Peg-IFN-α2a and Peg-IFN-α2b). The pegylation results in modified properties including sustained absorption/exposure and prolonged half-life, allowing for administration once weekly.23 Peg-IFN-α2a (40 kD), 450 µg once weekly, compared with IFNα-2a, 9 MIU once daily, resulted in higher rates of hematologic and cytogenetic response and improved overall survival.24 Therapy with Peg-IFN-α2a was also safe and better tolerated than conventional IFN-α. In a randomized phase III study, weekly Peg-IFN-α2b (PegIntron, PegIFN) was compared with daily IFN-α2b. The adverse events, efficacy and safety profiles were comparable.25 Thus, Peg-IFN-α represented an excellent candidate for clinical development in CML.

OVERVIEW OF PEGIFN: SAFETY (COMPILED FROM INVESTIGATOR’S BROCHURE)

PegIFN has been used for the treatment of chronic hepatitis C (CHC), HIV-1, CML, solid tumors (including melanoma and renal cell cancer), and multiple sclerosis (MS). Dosing exposure has ranged from 0.035 to 9.0 µg/kg/week. Treatment duration has been as long as 1 year in CHC, CML, and solid tumors, and as long as 6 months in MS. The safety profile of PegIFN appears to be similar across all indications and consistent with the safety profile of IFN. The most frequent reported adverse events with PegIFN are headache, fatigue, fever, myalgia, and arthralgia. There appears to be an interferon dose-independent increase in the incidence of most of the flu-like effects; prophylactic paracetamol may be useful. Most patients experience degrees of these problems which frequently wane during treatment. Injection site inflammation and injection site reaction is common with PegIFN, but it is generally mild to moderate and not treatment limiting. Psychiatric events including depression, suicidal ideation, psychosis, confusion and
asthenia are recognized problems with IFN. Autoimmune phenomena including mostly mild thyroid
dysfunction, pneumonitis (rare and sensitive to steroid treatment), and Vogt-Koyanaga-Hanada
syndrome, a rare granulomatous disorder of eyes, auditory system, meninges, and skin may be observed.
In oncology clinical trials, in which a dose of 6.0 µg/kg/week was administered, Grade 3 elevations in
hepatic enzymes (>5 to 20 times the upper limit of normal) have been observed. Dose-related decreases
in leukocyte, neutrophil, and platelet counts are associated with both PegIFN and IFN. In general these
side effects are reversible upon dose reduction or interruption. Lung infiltrates and dyspnea has been
reported with PegIFN. The investigators brochure (IB) for PegIntron is available under study documents

Overview of Pegylated IFN and Imatinib Combination Treatment in CML including safety.
Italian GIMEMA Working Party on CML has twice reported their phase II, open, single arm upfront study in
chronic phase CML with imatinib plus pegIFN. After two years 87 % of the 76 patients had discontinued
pegIFN, mainly due to toxicity, both hematological and non-hematological. The cytogenetic and molecular
responses at five years were excellent. A possible additional benefit from pegylated IFN was unassessable
due to low patient compliance. The intended dosages of pegylated IFN-α were 50, 100 and 150 µg weekly
and in practice too high (the average administered dose was only 33µg weekly), and lead to
discontinuation of this therapy.26;27 These results indicate that combination therapy of imatinib and
pegIFN, although attractive biologically, may be difficult to give in practice due to side effects. Probably
patients will only tolerate low doses of pegIFN combined with imatinib, a lesson for future studies.
The German CML IV Study was a randomized controlled comparison in newly diagnosed CP CML patients
of imatinib versus imatinib + conventional IFN-α versus imatinib + low-dose cytarabine (LDAC) or
Imatinib 800mg (or maximally tolerated dose (MTD)).28 No clear effect of IFN was seen, maybe
attributable to non-pegylated IFN formulation.
The French SPIRIT study has randomised 636 patients to four treatment arms: Imatinib 400mg/d,
Imatinib 600mg/d, Imatinib 400mg/d + LDAC and imatinib 400mg/d + pegylated IFN-α2a (Pegasys,
Roche) 90 µg/w.4 Although 46 % stopped Pegasys for toxicity reasons within 12 months, the IFN arm had
faster cytogenetic and molecular responses at 6 and 12 months and was the superior arm in the study.
Rates of MMR at 24 months were 71% and 48% for imatinib+Pegasys vs standard arm (p<.0001).
Interestingly, the dose was modified to 45 µg/week because of tolerability problems (actual administered
dose was 54 µg/w). A secondary analysis of the French study shows no difference in efficacy in the cohort
who received 45 vs 90 µg/week (Abstract 456, ASH 2011). Interestingly, the combination yielded more
complete molecular responses than standard arm treatment. Particular safety issues with the combination
treatment were hematological toxicity, in particular neutropenia, and various non-hematological toxicities,
such as bone pain, rash and fatigue. No apparent increase in liver toxicity was seen.
The NCMLSG randomized 112 newly diagnosed patients to imatinib 400mg OD +/-pegIFN (NordCML002).5
We aimed for a dose of 50µg weekly in low or intermediate Sokal risk group. We found that the
combination cohort achieved a very high molecular response rate at 12 months (82% vs 54% in the
standard arm), the main endpoint of the trial (p=0.002). Our clinical impression was that pegylated-IFN treatment was difficult to tolerate, similar to reports from the Italian and the French studies. An average dose of 42 µg was achieved in the 39% of the patients who continued the full scheduled treatment (12 months), and the average dose was only 24µg/w if the whole cohort was counted. In subgroup analysis, a duration of pegylated-IFN of >3 months appeared to be enough to achieve the effect, in analogy to the French SPIRIT study. Hematological toxicity, in particular neutropenia, and various non-hematological toxicities, such as bone pain, rash and fatigue caused discontinuation of pegIFN treatment. No serious neuropsychiatric side effects occurred, but 6% of patients refused to continue pegIFN treatment.

OVERVIEW OF PEGYLATED INTERFERON + 2TKIs INCLUDING SAFETY

There is one phase II study of the combination of nilotinib and Pegylated IFN-α2b (Pegasys) in CP-CML at diagnosis in France. Verbal communication from our French colleagues has indicated a high frequency of hematological toxicity. In the French study, patients take 2-3 times the PegIFN dose we plan in this study, and they also gave PegIFN during the first 3 months, which we have avoided because of expected hematological toxicity. The French colleagues now plan to amend their study to lower the dose.

There are no reported formal studies of the combination of dasatinib and PegIFN, but a case report showed very good effect of combined dasatinib+PegIFN in a patient with a double mutation.29

STUDY RATIONALE AND PURPOSE

The tyrosine kinase inhibitor (TKI) imatinib has dramatically improved the outcome for patients with chronic myeloid leukemia (CML) in chronic phase (CP). In the pivotal IRIS trial, a complete cytogenetic response CCyR was observed in 69% of the imatinib-treated patients within 12 months, and among 80% of CCyR responders a further reduction to less than 0.1% major molecular response MMR was seen after a total of 4 years treatment.30 This confers a reduced risk of disease progression and prolonged overall survival. Continued treatment with imatinib can further reduce the leukemic clone, in some patients down to levels undetectable by sensitive, quantitative PCR, so called “complete molecular response” (CMR), corresponding to at least a 4 to 5 log10 (MR4.0-5.0) reduction versus a standardized baseline derived from the initial IRIS study. Based on this and other studies, treatment recommendations (e.g from the European Leukemia Net and guidelines in each Nordic country) have been published.31 The mainstay of present treatment is standardized molecular and cytogenetic monitoring of the treatment response. According to these guidelines patients can be divided into “failure”, “suboptimal” and “optimal” categories based on speed of response at defined milestones. 5 years after debut about 40% of patients need to change treatment based on intolerance and poor response, mainly changing to 2TKIs.32

Recent data in newly diagnosed CML-CP patients, indicate that 2nd generation TKIs (2TKI) nilotinib and dasatinib are more effective than imatinib inducing MMR and MR4.5.1,2 In the DASISION trial after 12 months of treatment for dasatinib and imatinib respectively 83% vs 72 % CCgR, 46% vs 28% MMR was
observed. In the whole available cohort 13% vs 7% MR4.5 was observed, all figures statistically different.1

About 40% of patients who have achieved a sustained CMR for 2 years before stopping imatinib treatment also achieved prolonged freedom from relapse.3 This bears promise that improved treatment may increase the fraction of patients who can stop treatment, hopefully permanently, representing operational cure at least in a fraction of patients.

PegIFN has a prominent immunomodulatory mode of action and combined with imatinib improves treatment results compared to imatinib monotherapy.4;5 This advantage translates into higher MR rates (both MR 4.0 and MR4.5), and potentially future "cure". The improvement in treatment results of 2TKIs on one side and the combination of imatinib + PegIFN on the other appears to be similar in magnitude compared to standard imatinib treatment. Combining a 2TKI such as dasatinib and PegIFN is therefore logical as an attempt to improve treatment results.

Both dasatinib and PegIFN affect CML in two possible ways. Both agents affect tumor cell proliferation, but they also affect the immune system. The development of clonal LGL lymphocytosis in dasatinib-treated patients is associated with improved responses, but also with the development of lymphocytic pleuritis and pericarditis.20 The possibility of augmented CML immunity has also been discussed. 19;33;34 The exact mechanism of effect of IFN in CML is unclear. It has been suggested that the effects are mostly immune-related as IFN a key mediator of innate immunity responses.35;36 The recent discovery that IFN may cause cycling of stem cells has caught attention.37 There is ample in vitro evidence that primitive cells with putative stem cell characteristics are insensitive to TKIs.38 Cell cycling is likely to render stem cells sensitive to TKIs, forming a possibility for synergistic action.

The mechanisms of action of PegIFN and dasatinib are clearly different, hence a potential for additive or synergistic effects is available. Although previously not tested in combination in clinical trials, both drugs are approved. Adding a low dose of PegIntron to a very potent and immunostimulatory 2nd generation TKI dasatinib has the potential to increase adverse effects and a careful safety follow-up is warranted. However, as depicted above, also superior treatment responses and the chance for cure with the combination therapy are likely. We will also compare the data from this study with the data from the DASISION and NordCML006 study to estimate the additional effect of PegIFN.

OBJECTIVES AND ENDPOINTS OF THE STUDY

PRIMARY OBJECTIVES:
To study the efficacy and safety of combination treatment in newly diagnosed CML patients in chronic phase. A later phase III randomized study may be warranted if MMR (major molecular response, defined as ≤0.1% BCR-ABL1 on the International Scale) rates after 12 months are better than in the DASISION study (>46%) and if toxicity of the combination is not prohibitive.
SECONDARY OBJECTIVES:
Efficacy and toxicity, also compared to the DASISION registration study of dasatinib in 1st line treatment. Adherence to PegIFN. Disease progression. Quality of life. Effect of tumour burden in stem cells on treatment outcome. To perform immunological and other lab studies to explain effects and toxicity, and to attempt to identify biomarkers for response.

PRIMARY ENDPOINT:

*Safety endpoint in run-in phase (first 15 patients):* Study stops if four out of the first five, six of the first 10 or 8 of the first 15 patients experience grade IV hematological toxicity, grade III non-hematological toxicity or grade II serosal effusions during first 6 months of treatment. Data will be evaluated by an independent safety committee.

*Whole study efficacy endpoint:* Rate of MMR after 12 months.

SECONDARY ENDPOINTS:

1. Rate of CCgR after 3, 6, 12 and 18 months
2. Rate of MMR after 3, 6, 15, 18, and 24 months
3. Rate of MR4.0 and MR4.5 after 3, 6, 12, 15, 18, and 24 months
4. Rate of grade II non-hematological toxicity that poses a clinical problem (duration more than one month in spite of symptomatic treatment)
5. Rate of grade III hematological toxicity
6. Overall survival
7. Rate of patients who complete 3, 6 and 12 months of PegIFN treatment
8. QoL at 0, 3, 6, 12 and 18 months
9. Progression to advanced disease phase.
10. Comparison of safety and efficacy variables with historical cohorts from Nord CML006 and NordCML002
11. **Biomarkers** of response, failure and toxicity.
   - Fraction of leukemic cells in the stem cell (Ph+ CD34+CD38- cells) and progenitor cell (Ph+ CD34+CD38+) compartments at debut and 4 weeks of dasatinib treatment.
   - Lymphocyte subpopulations at 0, 3 and 6 months by flow cytometry assay: Enumeration of CD4+ and CD8+ T-cells, NK-cells, NKT-cells, B-cells and regulatory T-cells. Correlation of these subpopulations to response.
   - Functional assays of lymphocytes: Cytotoxicity of NK-cells. IFN-γ production by T cells after stimulation. Granzyme B staining for evaluating the cytotoxic potential of T-cells. Functional assays will be performed both from pre- and post-drug samples.
   - Clonality: TCR γδ rearrangements by PCR
**STUDY DESIGN**

Single arm, open label study of a combination of dasatinib 100 mg OD and PegIFN in patients with newly diagnosed CML See Figure 1 for a depiction of the design

**INCLUSION CRITERIA:**

1. Age 18-70 years of age
2. Diagnosis of chronic myeloid leukemia in chronic phase (CML-CP) associated with BCR-ABL1 quantifiable by RQ-PCR (IS)
3. No other current or planned anti-leukemia therapies excluding hydroxyurea treatment for up to two months.
4. ECOG Performance status 0, 1, or 2
5. Adequate organ function as defined by: Total bilirubin < 1.5 x ULN in absence of Gilbert genotype; ASAT and ALAT < 2.5 x ULN. Creatinine<2xULN. Potassium, magnesium and phosphate not below LLN.

6. Life expectancy of more than 12 months in the absence of any intervention

7. Patient has given written informed consent to participate in the study

EXCLUSION CRITERIA:

1. Prior accelerated phase or blast crisis

2. Uncontrolled or significant cardiovascular disease, including any of the following:
   a. A myocardial infarction within 6 months
   b. Uncontrolled angina within 3 months
   c. Congestive heart failure within 3 months
   d. Diagnosed or suspected congenital long QT syndrome
   e. Any history of clinically significant ventricular arrhythmias (such as ventricular tachycardia, ventricular fibrillation, or Torsades de Pointe)
   f. Prolonged QTcF interval > 450 msec on pre-entry ECG

3. Atypical BCR-ABL1 transcript not quantifiable by RQ-PCR.

4. Another primary malignant disease, which requires systemic treatment (chemotherapy or radiation)

5. Severe and/or life-threatening medical disease including acute liver disease and cirrhosis

6. History of significant congenital or acquired bleeding disorder unrelated to cancer

7. Impairment of gastrointestinal (GI) function or GI disease that may significantly alter the absorption of dasatinib

8. Patients actively receiving therapy with strong CYP3A4 inhibitors and the treatment cannot be either discontinued or switched to a different medication prior to starting study drug

9. Patients who are currently receiving treatment with any medications that have the potential to prolong the QT interval and the treatment cannot be either discontinued or switched to a different medication prior to starting study drug

10. Previous history of pericarditis or pleuritis

11. Female patients who are: pregnant, breast feeding or potentially fertile without a negative pregnancy test prior to baseline or unwilling to use contraception on trial

12. History of non-compliance, abuse of alcohol, illicit drugs, severe psychiatric disorders or other inability to grant informed consent.


14.

15. Hypersensitivity to any interferon preparation;
16. Autoimmune hepatitis or a history of autoimmune disease;
17. Pre-existing thyroid disease unless it can be controlled with conventional treatment;
18. Epilepsy and/or compromised central nervous system (CNS) function;
19. HCV/HIV patients

Re-screening: Patients who did not meet one or more inclusion or exclusion criteria may be re-screened for this study at a later time if the medical condition is transient and appropriately treated.

INFORMED CONSENT PROCEDURES

Investigators must ensure that patients are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical studies in which they volunteer to participate. Freely given written informed consent must be obtained from every subject prior to clinical study participation. If the patient has undergone cytogenetic evaluation or BCR-ABL1 RQ-PCR as part of the diagnostic workup before diagnosis and before study participation was considered or could be discussed, information from these procedures may be used for study records. This saves resources and discomfort (e.g. bone marrow punctures).

NCMLSG will provide the investigator with an informed consent form (ICF) for each participating country, appropriately approved by relevant authorities. The investigator should allow time necessary for the patient to ask about the details of the study, subsequently the ICF must be signed and personally dated by the subject and by the person who conducted the informed consent discussion. The patient should receive a copy of the signed informed consent and any other written information provided to study patients prior to subject's participation in the study.

UPDATE OF INFORMED CONSENT

The informed consent form and any other information provided to patients, will be revised whenever important new information becomes available that is relevant to the subject's consent. A revised ICF should receive EC approval prior to use. The investigator or someone he/she designates must see to that the subject is properly informed about the changes. This communication should be documented by signature of patient and informing staff on the revised ICF.

TREATMENT

INVESTIGATIONAL AND CONTROL DRUGS
Investigational treatment: Dasatinib combined with PegIFN (PegIntron)

Study drug: Dasatinib and PegIFN

**How supplied**

- Dasatinib will be supplied by Bristol Myers-Squibb
- Pegylated interferon (PegIFN-alfa2b) will be supplied by Merck

**Preparation and storage of Dasatinib**

- Dasatinib will be supplied to the Hospital Pharmacy in Trondheim, Norway

**Preparation and storage of pegylated interferon**

- PegIFN will be supplied in a refrigerated manner to the Hospital Pharmacy in Trondheim, Norway.

**Distribution of study drug**

The Trondheim pharmacy will distribute to national pharmacies in FI, SE and DK. These pharmacies will upon requisition from investigators send labeled study drug to the individual investigators or patients. Dasatinib will be sent at room temperature, Peg IFN in refrigerated packs. This means that the drugs must be sent in separate packs with individual temperature requirements

**Patient numbering**

Each patient in this study will be uniquely identified by a hospital identification number and a patient number (example 11001-703, i.e. hospital 11001 Karolinska sjukhuset, Stockholm -7 for NordCML007-and pat #03 in the study). Patient number will increase numerically according to sequence of inclusion in the whole study and will be assigned via WebCRF, CTU, NTNU, Trondheim, Norway. Once assigned to a patient, the subject number will not be reused. If the patient fails to be included for any reason, the center and subject number and the reason for non-inclusion will be entered on the Screening Log sheet.

**Patient treatment**

**Study treatment, study drug and administration**

Patients will be treated on-study for up to 24 months or until treatment failure or unmanageable toxicity. The “stabilizing phase” covers the first three months of the study treatment with daily dasatinib 100 mg OD. The “combination phase” ensues with continued daily dasatinib 100 mg OD combined with pegylated interferon 15µg weekly for three months up to the 6 month time point. If the patient tolerates the combination (i.e no grade 3-4 hematological toxicity or grade 2 non-hematological toxicity), a dose increase to PegIFN 25 µg weekly is attempted and continued until month 15 when PegIFN is discontinued. Thereafter dasatinib 100mg is continued until month 24.
It is underlined that the visit schedule is minimal and additional check-up of patient well being is necessary, particularly during the first 6 months of study. In the first 6 months hematology samples should be taken at 10-14-day intervals until CHR, and when PegIFN is added, we advice contacts on practical questions and subjective side effects on a weekly to two-weekly basis initially. The practical implementation of this is left to the investigator and may be carried out by doctors or nurses as seen fit. Telephone consultations and blood samples taken in primary care may be adequate. The outlined study visits must be at investigator’s clinic.

If concerns regarding the quality or appearance of study drugs arise, do not dispense it and contact the sponsor immediately via the CTU (University of Trondheim). Study drug should be stored in a secure area according to local regulations. PegIntron should be stored refrigerated at +4 degrees Celcius, and dasatinib should be stored at room temperature (excursions from 15-30 degrees Celcius). It is the responsibility of the investigator to ensure that study medication is only dispensed to study patients. The study medication must be dispensed only from official study sites by authorized personnel according to local regulations.

PACKAGING, LABELING AND INSTRUCTIONS TO PATIENTS

**Dasatinib**

Dasatinib will be labeled in open-label fashion. Each bottle will be labeled The label carries description of the contents, sponsor details including contact, and instructions on product use. Study medications will be packaged as follows:

- BMS-354825-03 20 mg film coated tablets, 30 tabs/bottle
- BMS-354825-03 50 mg film coated tablets, 30 tabs/bottle

Dasatinib should be ingested once daily, independently of meals. The capsules should be swallowed whole with water. All patients must avoid grapefruit and pomegranate juice during the study. Vomited doses should not be repeated. Since dasatinib has immediate effects on blood counts including platelets and the study has pharmacodynamic assessments (proteomics), patients should be advised not to take dasatinib the last 8 hours before blood sampling, and delay ingestion of dasatinib until later the same day.

**PegIFN (PegIntron)**

PegIFN will be labeled in open-label fashion. PegIFN will be provided in vials containing 50 µg of dry substance, to be diluted into 0,5 ml of sterile water. Each bottle will be labeled.
The label contains Description of the contents, sponsor details including contact and instructions on the use of product.

The patients will be instructed by study staff how to perform this and how to dose the study drug. The standard dose will be 15 µg/week, ie 0.15 ml of solution. In patients who tolerate the combination, a dose increase to 25 µg/week (0.25 ml) will be given from month 6 and onwards until month 15. In addition, a written dosing instruction based on the package insert will be provided for patient education together with the patient dosing booklet.

The patients will receive a cooling bag to ensure a temperature of approximately 4 degrees Celcius during transport (e.g between the clinic and home or during travel). Patients will be instructed to keep PegIFN refrigerated and study drug will be dispensed for 3-month periods. Patients will be instructed to bring their cooling bags to visits on month 6 and 9, for correct dispensation of PegIFN. Patients will be instructed to take 1g of paracetamol with PegIFN injections to allievate flu-like symptoms and may ingest up to 2g of Paracetamol each of the following 2 days.

**ADJUSTMENT OF STUDY DRUG DOSE (GENERAL PRINCIPLES)**

This study will use the CTCAE (NCI Common Terminology Criteria for Adverse Events) versions 3.0 for toxicity and adverse event reporting. It can be downloaded from [http://ctep.info.nih.gov/reporting/ctc.html](http://ctep.info.nih.gov/reporting/ctc.html) and are available in the WebCRF as a study-related document.

Adverse events occurring in the period between inclusion and three months should be attributed to dasatinib, and later occurring side effects to PegIFN or the combination. The patient’s status at Month 3 visit serves as baseline for toxicity attributable to dasatinib. AEs occurring after that should be attributed to PegIFN if not an AE typically related to dasatinib such as pleural effusion, pericarditis and pulmonary arterial hypertension (PAH) occurs (dasatinib-related serositis often occurs between Month 3 to Month 12).

Dose reductions should be made to the drug most likely to have caused the event. If unclear, PegIFN should be stopped before dasatinib. For patients who are unable to tolerate the protocol-specified dosing schedule, dose adjustments will be permitted in order to safely keep the patient on study drug. Dose changes must be recorded on the appropriate CRF. Along the treatment course adverse events may disappear and if the investigator believes that a dose increase will be tolerated at a later stage, the investigator should aim for the highest tolerable dose.

Motivation of patients and physicians to endure some discomfort is essential; the long-term aim is “cure” of the disease. Particularly flu-like symptoms (pyrexia, fatigue, bone
pain) frequently get better after some weeks and it is important not to give up too early (remember symptomatic treatment). Experimental and clinical data, however, indicate that it is better to give the patient some PegIFN than no PegIFN. Hence it is unwise to push the PegIFN dose so hard that the patient discontinues.

### Table 2: Dasatinib dose levels:

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>+1</td>
<td>140 mg OD</td>
</tr>
<tr>
<td>0</td>
<td>100 mg OD</td>
</tr>
<tr>
<td>-1</td>
<td>70 mg OD</td>
</tr>
<tr>
<td>-2</td>
<td>50 mg OD</td>
</tr>
</tbody>
</table>

### Table 3: PegIFN dose levels:

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>+1</td>
<td>25µg/week</td>
</tr>
<tr>
<td>0</td>
<td>15µg/week</td>
</tr>
<tr>
<td>-1</td>
<td>15µg/ every 2nd week</td>
</tr>
</tbody>
</table>

### Hematologic AEs

The classification of hematological toxicity is found in Table 4. It is underlined that the experience from previous combination studies of TKIs and PegIFN is that the combination induces more grade 3-4 neutropenia than TKI alone. Giving growth factors like G-CSF and erythropoiesis-stimulating agents such as erythropoietin is allowed and encouraged to maintain the dose of the study drugs. It is underlined that dasatinib has a mild platelet inhibitory function and it is important to exclude occult bleeds (e.g. GI tract hemorrhages) as cause of anemia.

### Table 4: Classification of hematological AEs (x10^9)

<table>
<thead>
<tr>
<th>Grade</th>
<th>WBC</th>
<th>Neutrophil</th>
<th>Platelet</th>
<th>Hemoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;LLN-3.0</td>
<td>&lt;LLN-1.5</td>
<td>&lt;LLN-75</td>
<td>LLN-100g/L</td>
</tr>
<tr>
<td>2</td>
<td>&lt;3.0-2.0</td>
<td>&lt;1.5-1.0</td>
<td>&lt;75-50</td>
<td>80-99 g/L</td>
</tr>
<tr>
<td>3</td>
<td>&lt;2.0-1.0</td>
<td>&lt;1.0-0.5</td>
<td>&lt;50-25</td>
<td>65-79 g/L</td>
</tr>
<tr>
<td>4</td>
<td>&lt;1.0</td>
<td>&lt;0.5</td>
<td>&lt;25</td>
<td>&lt;65 g/L</td>
</tr>
</tbody>
</table>

### Dose Modification for Patients on Anticoagulants

For patients on treatment with anticoagulants (acetyl salicylic acid, clopidogrel, low molecular weight heparins and oral inhibitors of thrombin or factor Xa) the following guidelines will apply for thrombocytopenia: If platelets ≤ 75 x 10^9/L (grade 2), withhold treatment with attributable drug until platelets have increased > 100 x 10^9/L and resume.
treatment at same dose. If recurrence, please discuss with steering committee. Evaluate the need for anticoagulant.

**DOSE ESCALATION FOR DASATINIB**

Dasatinib dose may be escalated to 140 mg OD in patients with a slow response. This is defined as no CyR after 3 months, No PCyR after 6 months and No CCyR after 12 months. A mutation analysis should be performed in such cases and if a mutation is detected a dose increase to 140 mg OD may be attempted if the mutation may be sensitive to dasatinib. Patients with mutations should be discussed with PI or study coordinator. Mutations which are highly resistant to dasatinib, such as V299L, T315I, T315A, and F317L/V, are reason for discontinuation of dasatinib and alternative therapies including allogeneic stem cell transplantation.

**DASATINIB DOSE REDUCTION FOR CARDIAC AE**

Significant electric heart abnormalities, including significant ventricular or atrial tachyarrhythmias: stop dasatinib indefinitely

QTc prolongation up to 480 msec: stop dasatinib, correct any serum electrolyte abnormalities, check ECG weekly, and resume dasatinib only when QTc ≤450 msec in two consecutive tests, at 50 mg OD (max allowed dose).

QTc prolongation to 500 msec or more: stop dasatinib indefinitely check ECG as clinically required, and fill in immediately an SAE report form.

<table>
<thead>
<tr>
<th>Table 5. Dose changes</th>
<th>Dasatinib alone</th>
<th>Combination treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dasatinib</td>
<td>PegIFN</td>
</tr>
<tr>
<td><strong>Hematologic toxicity (use of recombinant G-CSF, erythropoietin and transfusion is encouraged and allowed)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1-2</td>
<td>Continue unchanged treatment</td>
<td></td>
</tr>
<tr>
<td>Grade 3-4</td>
<td>Hold therapy. Resume 100mg when &lt;Grade2</td>
<td>Hold therapy Resume same dose</td>
</tr>
<tr>
<td></td>
<td>If recurrence: Hold therapy until &lt; grade2. Resume 70mg</td>
<td>If recurrence: Hold therapy until &lt; grade2. Resume at one dose level lower</td>
</tr>
<tr>
<td></td>
<td>If recurrence at 70 mg. Hold therapy until &lt; grade2. Resume at 50mg</td>
<td>If recurrence Hold therapy until &lt; grade 2. Resume at 15 µg/ every 2nd week (lowest allowed dose)</td>
</tr>
<tr>
<td></td>
<td>If recurrence at 50 mg, discuss a lower dose with study steering committee</td>
<td>If recurrence: Discontinue PegIFN</td>
</tr>
</tbody>
</table>
### Non-hematological toxicity

<table>
<thead>
<tr>
<th>Grade</th>
<th>No dose change</th>
<th>No dose change</th>
<th>No dose change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>Hold therapy. Resume 100mg when &lt;Grade2</td>
<td>Hold therapy. Resume same dose</td>
<td>No dose change</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Hold therapy until &lt; grade2. Resume 70mg</td>
<td>Hold therapy until &lt; grade2. Resume at one dose level lower</td>
<td>Hold therapy. Resume same dose</td>
</tr>
<tr>
<td></td>
<td>If recurrence: Hold therapy until &lt; grade2. Resume 50mg</td>
<td>If recurrence: Hold therapy until &lt; grade2. Resume at 15 µg/ every 2nd week</td>
<td>If recurrence: Discontinue PegIFN</td>
</tr>
<tr>
<td></td>
<td>If recurrence at 50 mg, discuss a lower dose with study steering committee</td>
<td>Applicable if antileukemic effect is good</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>If recurrence: Hold therapy until &lt; grade2. Resume 70mg</td>
<td>If recurrence: Hold therapy until &lt; grade2. Resume at one dose level lower</td>
<td></td>
</tr>
<tr>
<td></td>
<td>If recurrence: Hold therapy until &lt; grade2. Resume 50mg</td>
<td>If recurrence: Hold therapy until &lt; grade2. Resume at 15 µg/ every 2nd week</td>
<td></td>
</tr>
<tr>
<td></td>
<td>If recurrence at 50 mg, discuss a lower dose with study steering committee</td>
<td>Applicable if antileukemic effect is good</td>
<td></td>
</tr>
<tr>
<td>Grade 4</td>
<td>Hold therapy until &lt; grade2. Resume 50mg</td>
<td>Hold therapy until &lt; grade2. Resume at 15 µg/ every 2nd week</td>
<td></td>
</tr>
<tr>
<td></td>
<td>If recurrence at 50 mg, discuss a lower dose with study steering committee</td>
<td>Applicable if antileukemic effect is good</td>
<td></td>
</tr>
</tbody>
</table>

### Neuropsychiatric AE

<table>
<thead>
<tr>
<th>Grade</th>
<th>No dose change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 2-3</td>
<td>Hold therapy until &lt; grade2. Resume at 15 µg/ every 2nd week</td>
</tr>
<tr>
<td>Grade 4</td>
<td>Discontinue PegIFN</td>
</tr>
</tbody>
</table>

### Pleural Effusion and Pulmonary Arterial Hypertension (PAH): See below

<table>
<thead>
<tr>
<th>No dose change</th>
</tr>
</thead>
<tbody>
<tr>
<td>See text below</td>
</tr>
</tbody>
</table>

**Dose modifications and management of pleural effusion**

A total 10-15% of patients will develop pleural effusions during dasatinib monotherapy, most during the first year of therapy. Incidence and severity of pleural effusions during dasatinib-PegIFN is unknown and caution is warranted. The management algorithm for pleural effusions is outline in the following figure:
DOSE MODIFICATIONS FOR PULMONARY ARTERIAL HYPERTENSION (PAH)

PAH has been reported as a rare AE in patients treated with dasatinib, but because of the potential severity of PAH it is important to have this differential diagnosis in mind. The most common symptom is shortness of breath, but chest pain and signs of right ventricular heart failure may be seen. Exertional fatigue, dizziness or syncope has been reported. Such symptoms are rarely caused by PAH and we suggest that one excludes differential diagnoses as infections, pleural effusion and cardiac conditions. If no other explanation of symptoms is found, a work-up for PAH should be started in cooperation with relevant specialists. An echocardiogram and right ventricular catherization for pulmonary arterial pressure assessment may be warranted. If PAH is detected and either needs therapy (grade 2) or is evaluated as symptomatic (Grade 3-4), dasatinib and PegIFN should be discontinued, the patient should go off study and receive relevant treatment for PAH and another TKI for CML.

CONCOMITANT MEDICATIONS AND INTERACTIONS

In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the patient are allowed, provided their use is documented in the patient
records and on the appropriate case report form. These include blood and platelet transfusions for patients with anemia and with thrombocytopenia.

The concomitant administration of other investigational drugs than PegIFN or dasatinib is **not** allowed.

The administration of any other anticancer agents including chemotherapy and biologic agents is **not** permitted. The use of therapeutic coumarin derivatives (i.e. warfarin, acenocoumarol, phenprocoumon) is strongly discouraged since warfarin is metabolized by CYP2C9 and CYP3A4. Low molecular weight heparin and heparin may be substituted for coumarin. Other medications for anticoagulation should be considered.

Dasatinib is a weak competitive inhibitor of CYP3A4, CYP2C8, CYP2C9, CYP2D6, *in vitro*, potentially increasing the concentrations of drugs eliminated by these enzymes. If administration of a CYP3A4 inhibitor cannot be avoided, contact a member of Study Management Committee and document the decision on the WebCRF. Coadministration of CYP3A4 inducers including St. John's Wort (johannesört, hypericum perforatum), could increase the metabolism of dasatinib, and thereby decrease serum concentrations and reduce exposure to dasatinib.

PegIFN: Results from a multiple-dose probe study assessing P450 substrates in chronic hepatitis C patients receiving once weekly PegIntron (1.5 μg/kg) for 4 weeks demonstrated an increase in activity of CYP2D6 and CYP2C8/9. No change in activity of CYP1A2, CYP3A4, or N-acetyltransferase was observed. Caution should be used when administering peginterferon alfa-2b with medicines metabolised by CYP2D6 and CYP2C8/9, especially those with narrow therapeutic window, such as warfarin, phenytoin (CYP2C9) and flecainide (CYP2D6).

**Common CYP3A4 substrates**
The following lists describe medications which are common CYP3A4 substrates. The serum concentration of these drugs may be increased by dasatinib and PegIFN.

- **Macrolide antibiotics:** clarithromycin, erythromycin, but **NOT** azithromycin
- **Anti-arrhythmics:** quinidine
- **Benzodiazepines:** alprazolam, diazepam, midazolam, triazolam
- **Immune modulators:** cyclosporine, tacrolimus sirolimus
- **HIV antivirals:** indinavir, nelfinavir, ritonavir, saquinavir
- **Antihistamines:** astemizole, chlorpheniramine, terfenidine
- **Calcium channel blockers:** amlodipine, diltiazem, felodipine, lercanidipine, nifedipine, nisoldipine, nitrendipine, verapamil
• HMG CoA reductase inhibitors: atorvastatin, cerivastatin, lovastatin, BUT NOT pravastatin or simvastatin
• Steroid (6beta-OH): estradiol, hydrocortisone, progesterone, testosterone
• Others: alfentanil, buspirone, cafergot, caffeine, cocaine, dapsone, codeine-N-demethylation, dexamethorphan, eplerenone, fentanyl, finasteride, imatinib, haloperidol, irinotecan, LAAM, lidocaine, methadone, odanestron, pimozide, propranolol, quinine, salmeterol, sildenafil,
• tamoxifen, taxol, trazodone, vincristine, zaleplon, zolpidem

**Inhibitors of CYP3A4**
The following lists describe medications and foods which are strong to moderate inhibitors of CYP3A4. This list should not be considered all inclusive. Consult individual drug labels for specific information on a compound’s propensity to inhibit CYP3A4. CYP3A4 inhibitors could decrease the metabolism of dasatinib and increase exposure to dasatinib

**Table App. 5: CYP3A4 Inhibitors**

<table>
<thead>
<tr>
<th>Strong CYP3A4 Inhibitors</th>
<th>Moderate CYP3A4 Inhibitors</th>
<th>Weak CYP3A4 Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 5-fold increase in AUC</td>
<td>≥ 2 but &lt; 5-fold increase in AUC</td>
<td>≥ 1.25 but &lt; 2-fold increase in AUC</td>
</tr>
<tr>
<td>atazanavir, clarithromycin, indinavir, itraconazole, ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycins</td>
<td>amprenavir, aprepitant, diltiazem, erythromycin, fluconazole, fosamprenavir, grapefruit juice(a), verapamil</td>
<td>cimetidine</td>
</tr>
</tbody>
</table>

In patients for whom CYP3A4 inducers are indicated, alternative agents with less enzyme induction potential should be considered. If administration of a CYP3A4 inducer cannot be avoided, contact a member of Study Management Committee and document the decision on the Comments CRF.

A comprehensive list of cytochrome P450 isoenzymes and CYP3A4 inhibitors and inducers may be found at [http://medicine.iupui.edu/flockhart](http://medicine.iupui.edu/flockhart)

Patients must be carefully monitored for toxicity.

**Medications associated with QT interval prolongation**
Medications associated with QT interval prolongation that are prohibited in this study include (drugs in frequent clinical use are in bold):
• quinidine, procainamide, disopyramide
• amiodarone, sotalol, ibutilide, dofetilide
• erythromycins, clarithromycin
• chlorpromazine, haloperidol, mesoridazine, thioridazine, pimozide
• cisapride, bepridil, droperidol, methadone, arsenic, chloroquine, domperidone,
• halofantrine, levomethadyl, pentamidine, sparfloxacin, lidoflazine.

Please see http://www.torsades.org/medical-pros/druglists/printable-drug-list.cfm
for a comprehensive list of agents that prolong the QT interval. In cases where administration of a QT prolonging agent cannot be avoided, please contact a member of Study Management Committee, and document the decision on the Comments CRF. In such cases an ECG must be obtained both 24 to 48 hours and one week after initiating the concomitant therapy.

Patients should restrict the use of over-the-counter and prescription medicines containing paracetamol, but administration of doses of up to 2g/day is allowed after PegIFN administration. All patients must avoid grapefruit and pomegranate juice during the study.

Patients on anticonvulsants should have regular monitoring of plasma concentration of these agents.

The routine use of systemic corticosteroid therapy is permitted.

Growth factors may be initiated with recurrent Grade 3 neutropenia. Erythropoietin is allowed.

Anti-emetics may be allowed if the patient has experienced > Grade 1 nausea or vomiting.

STUDY DRUG DISCONTINUATION

Patients who discontinue study drugs should not be considered off study if willing to allow continued recording of response data, information about current CML treatment and important medical events including death. Data from such patients should be recorded and followed for the full planned 24 months and included in the safety and intention-to-treat populations. If patient or investigator discontinues PegIFN prematurely, a final study visit should be performed as soon as possible with blood counts, blood chemistry and sampling for lab studies.

END OF STUDY

After 24 months. Subsequent treatment will be at the discretion of the treating physician. If the patient has benefited from treatment, dasatinib will be provided free as study drug until dasatinib is reimbursed in first line use in the administrative region or
country where the patient lives. We will follow patient response by RQ-PCR, survival and progression to advanced phase until 5 years have passed.

**Discontinuation from Study**

Patients may be taken off the study prematurely for one of the following reasons:

- adverse event(s)
- abnormal laboratory value(s)
- abnormal test procedure result(s)
- treatment failure as defined above
- pregnancy
- Severe protocol violation
- consent withdrawn
- lost to follow-up
- administrative problems
- death

**Definitions of Risk, Disease Phase and Response.**

**Disease Risk Definition**

Sokal and EUTOS score at disease debut will be entered into the WebCRF disease history section. Calculators for these risk scores may be found at [www.roc.se](http://www.roc.se).

**Definitions of Advanced Phase CML:**

*Accelerated phase (AP) CML* will be defined by the presence of at least one of the following:

- The presence of ≥ 15%, but < 30% blasts in the blood or bone marrow
- At least 30% blasts plus promyelocytes in the blood or bone marrow
- At least 20% peripheral basophils
- Thrombocytopenia (fewer than 100,000 platelets/mm3) unrelated to treatment.

*Blast phase (BP) CML* will be defined by the presence of at least one of the following:

- At least 30% blasts in the blood or bone marrow
- Extramedullary involvement (e.g. chloroma) (heptaosplenomegaly excluded)

All the cases who do not meet any of the above criteria are defined as chronic phase.

**Progression-Free Survival (PFS)**

Progression-free survival is defined as the time from the date of randomization to the date of earliest progression-defining event (limited to transformation to blast crisis,
accelerated phase disease, or death from any cause).

**Event-free survival**
Event-free survival is defined as the time from date of randomization to the first occurrence of any of the following: loss of CHR, loss of CCyR, loss of MMR, death from any cause during treatment, progression to the accelerated phase or blast crisis of CML, whichever is earlier.

**Overall survival**
Overall survival is defined as the time from the date of inclusion to the date of death.

**Hematologic response (HR)**
Complete hematological response (CHR) if all the following criteria are met for more than one month
- WBC <10x10^9/L
- Differential without myelocytes, promyelocytes and myeloblasts
- Platelets < 450x10^9/L
- Spleen not palpable

**Cytogenetic response (CyR):**
The CyR will be evaluated with standard G-banding of BM aspirates within 6 weeks prior to randomization, at 3, 6, and 12 months. If the patient has not achieved a MMR (see below) by month 12, BM-aspirate and G-banding should be performed every 6 months until confirmed MMR is achieved. A minimum of 20 metaphases should be analyzed to be able to assess whether a complete cytogenetic response (CCyR, 0% Ph+ cells) has been achieved. Metaphase FISH may be used as a substitute to G-banding, or if necessary due to cryptic Ph translocations that are undetectable by G-banding.
A partial cytogenetic response (PCyR) is defined as 1%-35% Ph+ metaphases, a minor cytogenetic response (mCyR) as 36-65% Ph+ cells, a minimal cytogenetic response as 66-95% Ph+ cells. Samples with >95% Ph+ metaphases are considered as having no cytogenetic response.
If a sufficient number of marrow cell metaphases cannot be obtained, the definition of CCyR may be based on interphase FISH of blood or bone marrow cells, provided that it is performed with \( BCR-ABL1 \) extrasignal or dual color/dual fusion probes, and that at least 200 nuclei are scored. Using interphase FISH, CCyR can be assigned if the percentage of \( BCR-ABL1 \) positive cells is <1%. 

33
**Molecular response (MR).**
MR will be assessed by real-time quantitative reverse transcriptase polymerase chain reaction (RQ-PCR) performed on cDNA obtained from 10-20 ml (see table) of whole buffy coat blood leukocytes after red cell lysis. The relationship between *BCR-ABL1* and the measured control gene(s) should then be calculated as a percentage and expressed on the international Scale (IS) using a conversion factor, as previously described.41;42
As a minimum, the MR report should include the copy number (CN) of *BCR-ABL1* and the CN of the control gene(s) (may be reported as an average of several control genes), as well as the calculated percentage of *BCR-ABL1* relative to the control genes, expressed on IS. In this study, MR should be performed in a lab participating in quality rounds for IS measurements. A working group in NCMLSG will identify labs that provide an adequate standard for study purposes and participation in this study. National representatives within this group will when needed initiate quality checks and evaluation rounds.

- A molecular response with ≤0.1% *BCR-ABL1* IS is considered in major molecular response (MMR).
- MR$^{4.0}$ is defined as either (i) detectable disease ≤0.01% *BCR-ABL1* IS or (ii) undetectable disease in cDNA with ≥10,000 *ABL* transcripts (or ≥ 24,000 *GUS* transcripts).
- MR$^{4.5}$ is defined as either (i) detectable disease ≤0.0032% *BCR-ABL1* IS or (ii) undetectable disease in cDNA with ≥32,000 *ABL* transcripts (or ≥77,000 *GUS* transcripts).
- Samples with a total of <10,000 or <32,000 *ABL* transcripts (i.e. sum of the replicates if replicate analysis is performed) should be considered as inevaluable for MR$^{4.0}$ or MR$^{4.5}$, respectively. The same applies to samples with a total of < 24,000 or <77,000 *GUS* transcripts, respectively.

**Confirmed response**
Any response (Hematologic, cytogenetic or molecular) maintained over 2 consecutive measurements.

**Loss of response**
Loss of CHR: Increase of leukocytes > 10, and platelets > 450 if not for reactive causes. Reemergence of digitally palpable spleen. Reemergence of myelocytes, promyelocytes or blasts in peripheral blood.

Loss of CCyR: Confirmed detectable Ph+ metaphases in 2 consecutive samples.
Loss of MMR: A >5-fold rise (0.5 log) in *BCR-ABL1* transcripts from nadir to a level >0.1% (IS) in a patient who is in MMR. Loss of MMR must be confirmed in an independent sample.

**TREATMENT FAILURE**

Treatment failure is defined according to ELN recommendation (Baccarani JCO 2009):

- 3 months: No CyR (>95% Ph+ metaphases)
- 6 months: >65% Ph+ metephases
- 12 months: No PCyR, confirmed loss of CCyR or increase in *BCR-ABL* above 1%.

At all time points: Progression to AP/BP. The emergence of new mutations is considered treatment failure. Such patients should be discussed with study management.

**VISIT SCHEDULE AND ASSESSMENTS**

Table 6 lists all of the required assessments indicating with an “X” when visits are to be performed. All data obtained from these assessments must be present in the patient’s source documentation. Normal ranges of local laboratories will be used to describe deviations from upper or lower levels of normal (ULN, LLN) and AE grading according to CTCAE 3.0.

**SCREENING ACTIVITIES**

- **Screening:** Review medical history including CML diagnosis. Prior drug treatment and documentation disease history and treatments must be completed. Written informed consent must be obtained before any study specific medical procedures are performed. Study specific procedures will be performed within 14 days of study entry, with the exception of G-banding from bone marrow which is allowed within 2 months.

- **Re-screening:** Patients who did not meet one or more inclusion or exclusion criteria may be re-screened for this study at a later time if the medical condition is transient and appropriately treated.

**REQUIRED PROCEDURES**

- Signed informed consent
- Physical Examination
- Assessment of ECOG Performance Status
- Hematology including hemoglobin, total WBC count, platelet count, and a manual differential count including neutrophils, bands, lymphocytes, monocytes, eosinophils and basophils. A manual differential is required at debut and until a CHR has been achieved. There after an automated blood count is acceptable.
- Chemistries include creatinine, total bilirubin (direct or indirect if > 25), alkaline phosphatase (ALP), ASAT, ALAT, potassium, magnesium, and phosphorus
- Blood sample collection for shipment to PCR Laboratory
- Blood sample for lymphocyte studies (see appendix)
- Blood sample for proteomic studies (special buffer needed) (See appendix)
- Bone marrow cytogenetics
- Bone marrow stem cell sample (see appendix)
- Electrocardiogram (ECG)
- Chest X-ray
- Concurrent Medications: Educate patients on the importance of reporting all medications including over-the counter medications. Educate on medications that should be avoided explaining the potential for interaction.
- Dasatinib dosing and compliance. Educate patient on dosing administration.
- PegIFN: dosing, compliance. Educate patient about usual side effects of IFN, use of paracetamol to alleviate flu-like symptoms. Instruct patient about need for refrigeration of PegIFN, how to resuspend PegIFN and perform the injections.
Table 6. Visit evaluation schedule.

<table>
<thead>
<tr>
<th>Activity / Test</th>
<th>Screening/Baseline</th>
<th>Month 1,4,5</th>
<th>Month 3,6,9, 12,15,18, 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informed Consent</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inclusion/Exclusion Criteria</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical History</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical examination, body weight, height</td>
<td>X  X  X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECOG performance status</td>
<td>X  X  X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematology(^1)</td>
<td>X  X  X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood chemistry(^2)</td>
<td>X  X  X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy test, if applicable(^3)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood RQ-PCR for BCR-ABL(^4)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone marrow smear(^5)</td>
<td>X</td>
<td>M3, M6, M12</td>
<td>Later as needed</td>
</tr>
<tr>
<td>Cytogenetic exam (karyotyping)(^5)</td>
<td>X</td>
<td>M3, M6, M12</td>
<td>Later as needed</td>
</tr>
<tr>
<td>ECG</td>
<td>X</td>
<td>M1 only</td>
<td></td>
</tr>
<tr>
<td>QoL</td>
<td>X</td>
<td>M3, M6, M12</td>
<td>M18</td>
</tr>
<tr>
<td>Chest radiogram</td>
<td>X</td>
<td>When clinically indicated</td>
<td></td>
</tr>
<tr>
<td>Concomitant medications</td>
<td>X</td>
<td>Continuous</td>
<td></td>
</tr>
<tr>
<td>Dosing and compliance</td>
<td>X</td>
<td>Continuous</td>
<td></td>
</tr>
<tr>
<td>Adverse events</td>
<td>X</td>
<td>Continuous</td>
<td></td>
</tr>
<tr>
<td>Serious Adverse Events</td>
<td></td>
<td>Continuous</td>
<td></td>
</tr>
</tbody>
</table>

1) Hematology to include: Hb, Hct, total WBC count, platelets automated differential including neutrophils, lymphocytes, monocytes, eosinophils and basophils. Automated differential is permitted in patients with stable disease, but manual differential is strongly encouraged when a change in disease status is suspected. **Patients should be instructed not to take dasatinib the last 8 hours before blood sampling, i.e. delay taking the daily dose until after blood sampling**

2) Chemistries to include: creatinine, total bilirubin (direct or indirect), ALP, ASAT, ALAT, calcium, , potassium, magnesium, and phosphorus.

3) For potentially fertile women. A negative serum or urinary pregnancy test is required

4) 10 ml of PB required, but when patients have achieved MMR or better, 20 ml of PB is required for sensitivity reasons.
5) Karyotyping must be carried out until confirmed CCgR (i.e. 2 consecutive negative samples with minimally 20 metaphases). If CCgR is not confirmed by month 12, a cytogenetic examination must be performed every 6 months until confirmed CCgR.

<table>
<thead>
<tr>
<th>Immunology/stem cell substudies</th>
<th>On treatment visits</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Procedure:</strong> See Appendix 2, 3 and 5 for details</td>
<td><strong>Pre-treatment</strong></td>
</tr>
<tr>
<td><strong>Lymphocyte studies:</strong> Collection of PB sample (30ml heparinized blood) before drug intake</td>
<td>X</td>
</tr>
<tr>
<td><strong>Dasatinib plasma concentration</strong> and differential count (One 5ml EDTA tube) before and 1 h after dasatinib</td>
<td></td>
</tr>
<tr>
<td><strong>Phosphoflow:</strong> (5 ml citrate tube) before and 1 h after dasatinib</td>
<td></td>
</tr>
<tr>
<td><strong>Stem cell for flow:</strong> 10ml heparinized BM</td>
<td>X</td>
</tr>
<tr>
<td><strong>Stem cell for FISH:</strong> 30ml heparinized BMone marrow</td>
<td>X</td>
</tr>
</tbody>
</table>

**Samples for phosphoflow** must be taken before and 1h after intake of dasatinib. Store locally in -80°C freezer and ship in larger batches (on dry ice). See Appendix 5 for instruction in detail. If there are considerable problems for the patient (i.e long travel) to come to clinic on day 1 for phosphoflow, a baseline sample at the pretreatment visit is also very valuable.

**Stem cell assay:** If dry tap or poor yield in aspiration, send 30 ml of peripheral blood. See Appendix 2 on the scientific substudies.
INFORMATION TO BE COLLECTED ON SCREENING FAILURES
The demographics of patients who are screen failures, as well as the reason the patient did not qualify for the study will be captured in the Screening Log.

PATIENT DEMOGRAPHICS/OTHER BASELINE CHARACTERISTICS

INCLUSION/EXCLUSION CRITERIA
Patient eligibility is to be established by confirming all inclusion/exclusion criteria.

DEMOGRAPHICS
The patient’s year of birth, initials and sex, will be recorded on the WebCRF.

RELEVANT MEDICAL HISTORY/CURRENT MEDICAL CONDITIONS
Relevant medical history and current medical conditions,

PRIOR TREATMENTS
Prior doses and duration of hydroxyurea. Prior antineoplastic medications, radiotherapy, and surgeries, including surgical biopsies.

DISEASE HISTORY
Date of initial diagnosis of CML. Sokal and EUTOS score at debut through entering basophils, age, spleen size, PB blasts, and platelet count.

TREATMENTS
Compliance will be assessed by the investigator and/or study personnel at each visit. Patients will be instructed to bring back empty tablet boxes and used PegIFN syringes. The number of remaining tablets and used syringes should be counted and recorded. In addition information provided by the patient or written information in the patient medicine booklet will be used to assess compliance. The quantity of used drug will be captured in the medical record and WebCRF at each visit.

DOSAGE ADMINISTRATION RECORD
Total dose of dasatinib (in mg) and PegIFN (in µg) in each 3-month interval will be recorded in WebCRF. Any changes in dose including interruptions or reductions must be recorded in WebCRF.

CONCOMITANT MEDICATIONS/SIGNIFICANT NON-DRUG THERAPIES
All prescription medications and over-the-counter drugs including vitamins and blood transfusions taken within 30 days prior to the start of and throughout the study must be recorded on the Concomitant Medications/Non-Drug Therapies CRFs. Medication entries should include the trade name, the start and discontinuation dates and the reason for therapy. Growth factors such as G-CSF and erythropoiesis stimulating agents (ESA) are
allowed but their use must be annotated

**QUALITY OF LIFE (QoL)**
For assessment of QoL we will use the QLQ-C30 form. Paper forms will be handed out to patients at the relevant study visits. Data will be analysed by Dr AK Kvam, Oslo University Hospital and compared with treatments, toxicity and efficacy

**SURVIVAL INFORMATION**
All patients who enter the follow-up phase will be followed for survival and BCR-ABL 6-monthly for a total of 3 years as part of standard of care.

**COMMENTS**
All comments related to study conduct will be recorded in the Comments field of the CRF.

**PHYSICAL EXAMINATION, WEIGHT**
A physical examination will be performed according to the Visit schedule (see Table 6). Physical examination should include weight, spleen and liver size in cm below costal margin, and other relevant findings. Weight should be measured.

Report changes by physical examinations in patient medical record, and comment relevant findings in WebCRF

**PERFORMANCE STATUS**
Performance status will be recorded in the CRF according to table 5 and as defined by the ECOG criteria in Table 7.

<table>
<thead>
<tr>
<th>Grade</th>
<th>ECOG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Fully active, able to carry on all pre-disease performance without restriction</td>
</tr>
<tr>
<td>1</td>
<td>Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work</td>
</tr>
<tr>
<td>2</td>
<td>Ambulatory and capable of all self care but unable to carry out any work activities. Up and about more than 50% of waking hours</td>
</tr>
<tr>
<td>3</td>
<td>Capable of only limited self care, confined to bed or chair more than 50% of waking hours</td>
</tr>
<tr>
<td>4</td>
<td>Completely disabled. Cannot carry on any self care. Totally confined to bed or chair</td>
</tr>
<tr>
<td>5</td>
<td>Dead</td>
</tr>
</tbody>
</table>
**ELECTROCARDIOGRAM (ECG)**

All patients will have ECG assessments during the study. Patients will have ECGs to monitor QTc at screening/baseline and 1 months after start.

**CHEST RADIOGRAM**

A chest radiogram will be taken before study start and will be repeated when clinically indicated.

**BIOMARKER PROJECT AND BIOBANKING** *See Appendix*

**MUTATION TESTING**

It is recommended to perform mutational analysis as a standard of care as per ELN recommendations upon treatment failure, loss of CHR, loss of CCyR, loss of MMR and upon a rise in *BCR-ABL* transcripts of greater than 5-fold.

**SAFETY ASSESSMENTS**

Safety assessments will consist of evaluating adverse events and serious adverse events and concomitant medications/therapies used to treat them, laboratory parameters including hematology, chemistry, body weight, physical examinations, and ECG monitoring.

**ADVERSE EVENTS**

An adverse event (AE) is defined as any untoward medical occurrence or worsening of a pre-existing medical condition in a subject or clinical investigation subject administered an investigational (medicinal) product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

Adverse events will be assessed according to the Common Toxicity Criteria for Adverse Events (CTCAE) version 3.0, [http://ctep.cancer.gov/forms/CTCAEv3.pdf](http://ctep.cancer.gov/forms/CTCAEv3.pdf) and in “Study Documents” section in the WebCRF. The occurrence of adverse events should be sought by non-directive questioning of the patient at each visit during the study. Adverse events also may be detected when they are mentioned by the patient during or between visits or through physical examination, laboratory test, or other assessments. As far as possible, each adverse event should be evaluated to determine:

1. The severity grade (CTCAE grade 1-4)
2. Its relationship to dasatinib or PegIFN (suspected/not suspected)
3. Its duration (start and end dates or if continuing at final exam)
4. Action taken (medical treatments and dose modifications should be recorded.)
SERIOUS ADVERSE EVENTS

A serious AE (SAE) is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or causes prolongation of existing hospitalization (see note below for exceptions)
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect (note: reports of congenital anomalies/birth defects must also be reported on the Pregnancy Supplemental Form [see Section 9.6])
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g., medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)
- Overdose (accidental or intentional) defined as administration of > 100 µg of PegIFN in 7 days to a single patient Or >500 mg of dasatinib once or > 1500 mg/week).
- Pregnancy
- Cancer

The following hospitalizations are not considered SAEs:
- Admissions for a planned medical/surgical procedure
- Routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)

All SAEs whether related or unrelated to study drugs must be immediately reported by the investigator or designee to Trondheim CTU and to the country-specific MSD pharmacovigilance department in the country where the SAE occurred within 24 hours of becoming aware of the event. Safety reporting to BMS will be to their global pharmacovigilance department. CTU Trondheim sends copies of these faxes to all National investigators. Investigators or their designees must fill out the SAE form to be found under “study documents” in the WebCRF. Information must be faxed and the fax numbers to be used for
each country are written on the cover sheet of the SAE form. If only limited information is initially available, follow-up reports are required. The original SAE form must be kept on file at the study site.

**SUSAR**

Suspected unexpected serious adverse reactions (SUSARs) are serious events that are not listed in the Summary of Product Characteristics (SPC) and that the investigator identifies as related to study drug. Country specific SUSARs will be reported by study management to the country-specific Medicines Agency, the country-specific MSD Pharmacovigilance Department and BMS Global Pharmacovigilance Department (+1 609-818-3804 USA) within 7 days from notification of the event.

**Assignment of Adverse Event Intensity and Relationship to Investigational Product**

NCI CTCAE version 3.0 will be used to grade AEs. Grade 3 or grade 4 hematological AEs will be reported. Non-hematological AE grade 3 and 4, but also protracted and clinically relevant grade 2 AEs will be reported.

The relationship of AE and study drug (causality) will be classified as:

- **Certain**: There is a reasonable causal relationship between the investigational product and the AE. The event responds to withdrawal of investigational product (dechallenge), and recurs with rechallenge when clinically feasible.
- **Probable**: There is a reasonable causal relationship between the investigational product and the AE. The event responds to dechallenge. Rechallenge is not required.
- **Possible**: There is reasonable causal relationship between the investigational product and the AE. Dechallenge information is lacking or unclear.
- **Not likely**: There is a temporal relationship to investigational product administration, but there is not a reasonable causal relationship between the investigational product and the AE.
- **Not related**: There is not a temporal relationship to investigational product administration (too early, or late, or investigational product not taken), or there is a reasonable causal relationship between non-investigational product, concurrent disease, or circumstance and the AE.

**Pregnancy**

Potentially fertile women must use an effective method of birth control during the course of the study, in a manner such that risk of failure is minimized. It is the obligation of the investigator to particularly stress the consequences of pregnancy when informing a
potentially fertile woman about the study. Male participants have no restrictions in this concern (See ICF forms)

**REQUIREMENTS FOR PREGNANCY TESTING**
All potentially fertile women MUST have a negative pregnancy test (serum or urine). If the woman suspects pregnancy, a new pregnancy test will be performed. If positive, and the woman does not want to undergo a abortion, she must immediately stop intake of study drug and be excluded from the study. Pregnancy must be reported within 24 hs on the SAE form. The outcome of the pregnancy will be followed up to 2 years of age.

**SAFETY MONITORING COMMITTEE (SMC) AND INTERIM ANALYSIS.**
A safety interim analysis is planned after 15 patients have completed 6 months of study, i.e. 3 months on the combination. The data monitoring committee will make a recommendation as to whether the study should continue, stop, or be modified based on its findings. The SMC recommendation will be notified PI verbally and in written form as soon as possible after the meeting. Study stops if four out of the first five, six of the first 10 or eight of the first 15 patients experience grade IV hematological toxicity, grade III non-hematological toxicity or grade II serosal effusions during first 6 months of treatment. After 15 patients have completed 9 months on study, i.e. 6 months on PegIFN, the effect of the dose increase to 25 ug/w at Month 6 will be assessed, and a recommendation considering this dose increase will be made.

**DATA REVIEW AND DATA MANAGEMENT**

**DATA COLLECTION, DATABASE MANAGEMENT AND QUALITY CONTROL:**
Data will be entered into an internet-based CRF (WebCRF) designed and protected by the Dept of Applied Clinical Research (CTU), Norwegian University of Science and Technology, (NTNU), Trondheim, Norway. Chief of Department is Prof Sven M Carlsen. Appropriate passwords will be designated to the investigators for entry of data. Database management will be performed by the same institution.

**STATISTICAL METHODS AND DATA ANALYSIS**

**POPULATIONS FOR ANALYSIS**
Intention-to-treat and safety population all included patients who received at least one dose of study drug.
TREATMENTS (STUDY DRUG, CONCOMITANT THERAPIES, COMPLIANCE)

The duration of exposure to the study medication and dosing intensity taken by the patients will be summarized in the safety population. Concomitant medications and significant non-drug therapies prior to and after the start of the study drug will be summarized by the safety population.

PRIMARY ENDPOINT

Safety: The study stops if >50% of patients (i.e. 8 or more of 15) experience grade IV hematological toxicity, grade III non-hematological toxicity or grade 2 serositis (after 3 months of combination treatment).

Efficacy: To evaluate the rate of MMR 12 months after entering the study.

STATISTICAL HYPOTHESIS, MODEL, AND METHOD OF ANALYSIS

For toxicity: Frequencies of different grades and types of AE will be calculated and tabulated with similar data from NordCML002, NordCML006 and DASISION on the safety population.

For efficacy: The primary efficacy analysis will calculate the rate of patients in MMR by 12 months in the intention-to-treat population. Using the Fleming one-stage approach expecting a clinically meaningful difference to be minimally 23% increase in MMR rate (from 46% in DASISION to 69% in this study) to be clinically interesting. Dismissal of the null hypothesis (H₀; p<0.46 occurs if 69% or more of the patients achieve MMR, implying 23 or more out of 35 patients.

SECONDARY OBJECTIVES AND EXPLORATORY ANALYSIS

STATISTICAL HYPOTHESIS, MODEL, AND METHOD OF ANALYSIS

Analyses include calculating the rates of CCgR, MMR, MR4.0 and MR4.5 at 3, 6, 12, 18 and 24 months. The same will be done for best overall response at any time for CCyR, MCyR, CHR and MMR. For secondary and tertiary endpoints, the estimation of PFS, and OS, time to and duration of CCyR, MMR, MR4.0 and MR4.5 using the Kaplan-Meier product limit method.

Comparison with historical cohorts from DASISION, Nord CML002 and NordCML006 will be performed as: A) A tabulation of response data at 3,6,12, 15, 18 and 24 months in separate cohorts of imatinib alone, dasatinib alone, imatinib and PegIFN combination and dasatinib+PegIFN combination. B) A comparison of responses will be performed using
Cochran-Mantel-Haenszel (CMH) approach for the difference in rates between the two treatment arms along with their two-sided 95% CI. The comparison of times in CCyR, MMR, MR4.0 and MR4.5 will be conducted via a two-sided $\alpha=0.05$ stratified log-rank test. The median will be provided along with its 95% CI and the hazard ratio of the two treatment groups (along with its two-sided 95% CI) will also be provided. These analyses using historical data have purely exploratory purposes and will be used in evaluations concerning future trials.

Average dose of given PegIFN and dasatinib will be calculated at 3, 6, 9, 12, 15, 18 and 24 months. Fraction of patients able to continue planned study treatment will be calculated at the same time points.

**EXPLORATORY ANALYSIS**

Descriptive analysis for biomarkers of response 0, 3 and 6 months. The treatment cohort will be divided in “slow” (above median) and “fast” responder categories (at or under median) by molecular response at 3, 6 and 12 months. Patients will also be categorized into those who achieve MR4.0 or not at 12 and 24 months. Correlations between absolute values for molecular response, these categories and different biomarkers will be performed for exploratory purposes with the intent of selecting promising biomarkers for later validation. Difference in biomarker levels from 0-3 months is attributable to dasatinib. Difference in biomarker levels from 3 to 6 months is attributable to PegIFN.

**QoL:** Statistical analysis to be decided in cooperation with the QoL group at Oslo University Hospital (AK Kvam). It is reasonable to compare QoL parameters in the half of cohort with best response to the half with poorest response measured by MR. It is also natural to compare patients with side effects a certain level (i.e gr 3-4) or type (i.e. hematological or serositis) with the remainder. The analysis is exploratory.

**SAFETY PARAMETERS AND ANALYSES**

All the safety analyses will be based on the safety population. The assessment of safety will be based mainly on the frequency of adverse events and are descriptive.

**ADVERSE EVENTS (AE)**

All adverse events recorded during the study will be summarized. The incidence of treatment-emergent adverse events (new or worsening from baseline) will be summarized by system organ class, severity (based on CTCAE grades), type of adverse event, relation to the study drug by treatment group. Deaths reportable as SAEs and non-fatal serious
adverse events will be listed by patient and tabulated by type of adverse event and
treatment group.

INTERIM ANALYSIS

A safety analysis will be performed after 15 patients have been treated 6 months, i.e. 3
months on the dasatinib and PegIFN combination. Estimation of efficacy will also be
performed.

SAMPLE SIZE CALCULATION AND STATISTICAL POWER

Our sample size will form a good basis for power calculations and estimation of
therapeutic potential for a future phase III trial. To compare with historical cohorts (mainly
the registration study DASISION, but also NordCML002 and 006) we base the following
assumptions. With 35 patients one can detect a 23% difference in MMR rates with a
statistical power of 80%. This means 69% MMR compared to historical 46% in DASISION.
The absolute increase in MMR rate with pegylated interferon found in French SPIRIT and
NordCML002 (20% and 28%) is in this range, and the absolute MMR rate was ca. 60% and
82% respectively in these two studies (at month 15 post imatinib start). We think that such
a difference is a very possible outcome of the presently proposed study (i.e. >69% MMR
rate). Using the Fleming one-stage approach expecting 23% increase in MMR rate as a
clinically meaningful difference, by strict analysis 30 patients are needed for the efficacy
estimate. The null hypothesis should be dismissed if 19 patients or more achieve MMR. To
avoid problems with statistical power we have decided to include 35 patients, dismissing the
null hypothesis at 23 patients or more who achieve MMR.

ETHICAL ISSUES AND ADMINISTRATIVE PROCEDURES

ETHICAL CONSIDERATIONS
Patients who take part in this study will receive therapy with high expected efficacy against
their illness. It is possible and maybe likely that PegIFN will improve the responses
compared to dasatinib alone, and this trial aims to assess the effect for potential proper
randomized comparisons. It is likely that patients who achieve a deep molecular remission,
.i.e MR4.0 or better, may stop drug treatment long-term without relapse and this
combination regimen may increase the number of patients who achieve this therapeutic
goal. Both drugs have immunostimulatory properties, however, not overlapping. A
synergistic effect is thinkable both for efficacy and side effects. These concerns are reflected
in rigorous supervision of side effects. Investigators are all experienced in handling both
interferon and dasatinib individually. An interim evaluation by a safety committee of two senior hematologists is planned and in the event of unexpected severity or type of side effects the study will be stopped. After study, patients may continue dasatinib if they want, independently of reimbursement policy in their country.

GOOD CLINICAL PRACTICE
This study will be conducted in accordance with Good Clinical Practice (GCP), as defined by the International Conference on Harmonisation (ICH) and in accordance with the ethical principles underlying European Union Directive 2001/20/EC and the United States Code of Federal Regulations, Title 21, Part 50 (21CFR50). The study will be conducted in compliance with the protocol. The protocol and any amendments and the subject informed consent has to receive EC approval/favorable opinion before initiation of the study. Study personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective task(s).

ETHICS COMMITTEE (EC)
Before study initiation, the investigator must have written and dated approval/favorable opinion from the EC for the protocol, consent form and any other written information to be provided to patients. The investigator or sponsor should also provide the EC with a copy of the Investigator Brochure or product labeling, information to be provided to patients and any updates. The investigator or sponsor should provide the EC with reports, updates and other information (e.g., expedited safety reports, amendments, and administrative letters) according to regulatory requirements or institution procedures.

REGULATORY AND ETHICAL COMPLIANCE
This clinical study was designed and shall be implemented and reported in accordance with the protocol, the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations (including European Directive 2001/20/EC and US Code of Federal Regulations Title 21), and with the ethical principles laid down in the Declaration of Helsinki.

COMPLIANCE WITH THE PROTOCOL AND PROTOCOL REVISIONS
The study shall be conducted as described in this approved protocol. All revisions to the protocol must be performed by the NCMLSG. If the protocol is amended, the investigators may not implement any change before the EC has approved the change, except where
necessary to eliminate an immediate hazard(s) to study patients. Any significant deviation from the protocol must be documented in the individual patient’s CRF.

Investigational Site Training
At study start a training course will be provided. Training topics will include but are not limited to: GCP, AE reporting, study details and procedure, study documentation, informed consent, and enrollment of potentially fertile women.

Records Retention
The investigator must retain investigational product disposition records and source documents for the maximum period required by applicable regulations and guidelines, or institution procedures, or for the period specified by the sponsor, whichever is longer. If the investigator withdraws from the study (e.g., ending his/her employment), the records shall be transferred to a mutually agreed upon designee (e.g. another investigator). Notice of such transfer will be given to NCMLSG.

Case Report Forms (WebCRF)
All sites within this study will use electronic CRFs submit study data. The investigator will maintain a signature sheet to document signatures and initials of all persons authorized to make entries and/or corrections on CRFs. Electronic CRFs must be promptly and accurately completed by an investigator, subinvestigator or study nurse.

Investigational Product Records
It is the responsibility of the investigator to ensure that a current record of dasatinib and PegIFN disposition is maintained at each study site where investigational product is inventoried and disposed. Records or logs must comply with applicable regulations and guidelines and should include:

- amount received and placed in storage area
- amount currently in storage area
- label ID number or batch number and use date or expiry date
- dates and initials of person responsible for each investigational product inventory entry or movement
- amount dispensed to and returned by each subject, including unique subject identifiers
- amount transferred to another area/site for dispensing or storage
- non-study disposition (e.g., lost, wasted, broken)
- amount returned to the sponsor
- amount destroyed at study site, if applicable
- retain samples sent to third party for bioavailability/bioequivalence, if applicable

The log should for practical reasons be handled by the dispensing pharmacy. Investigational product dispensing record/inventory logs and copies of signed packing lists must be maintained at the investigational site. Batch numbers must be recorded in the drug accountability records.

**DRUG ACCOUNTABILITY**

The label ID number or batch number, date and amount dispensed of all tablets and PegIFN must be recorded on the drug accountability pages by investigator or study nurse. The subject must be instructed to return all unused study medications in the provided packaging at each subsequent visit. The investigator must be satisfied the subject returned or accounted for all unused medication before additional medication is dispensed. If the number of tablets used is substantially different from the number of tablets dispensed, the subject must be counseled on how study therapy should be taken. If such deviations persist, the investigator may consider discontinuing the subject for non-compliance.

**MONITORING**

Appointed monitors must be allowed to visit all study site locations periodically to assess the data quality and study integrity. On site they will review study records and directly compare them with source documents, discuss the conduct of the study with the investigator, and verify that the facilities remain acceptable. Data from all patients will be monitored by assigned monitors in all countries from source documents on site. A guideline for NCMLSG study monitoring is found in Appendix 4. To ensure reasonable cost-effectiveness for the monitoring, the study management may allow monitoring of copied source documents, if it is deemed unreasonable to monitor on site (i.e. in the end of the study) and other data have been entered in a credible and accurate manner from the same hospital.

**RETURN AND DESTRUCTION OF INVESTIGATIONAL PRODUCT**

Upon completion or termination of the study, all unused and/or partially used investigational product must be destroyed at the site in accordance with local and institutional guidelines.
References


**APPENDIX 1**

**PARTICIPATING HOSPITALS AND INVESTIGATORS:**

**Denmark (DK):**

Jesper Stentoft (National Coordinator [NC]) (Aarhus University Hospital),
Ole Weis Bjerrum (Rigshospitalet. Copenhagen).

**Finland (FI):**

Satu Mustjoki (NC), Kimmo Porkka, and Perttu Koskenvesa (all Dept of Hematology Helsinki University Hospital).

**Norway (NO):**

Tobias Gedde-Dahl (Oslo University Hospital)
Bjørn Tore Gjertsen (Haukeland, Bergen University Hospital)
Waleed Majeed (Stavanger University Hospital),
Franz Gruber (Tromsø, University Hospital of Northern Norway),
Henrik Hjorth-Hansen (St Olavs Hospital-Trondheim University Hospital)(NC).

**Sweden (SE):**

Ulla Olsson Strömberg (Uppsala University Hospital (Akademiska))(NC)
Johan Richter (Lund University Hospital),
Leif Stenke, Lotta Ohm and Sören Lehmann (Karolinska Hospital, Stockholm)
Berit Markevärn (Umeå University Hospita)
Mats Björeman (Örebro University Hospital)
Claes Malm (Linköping, University Hospital)
Anders Själander (Sundsvall County Hospital)
Kristina Myhr Eriksson (Sunderby Sjukhus, Luleå).

**PARTICIPATING PCR LABORATORIES**

**Denmark (DK):**

Aarhus: (Charlotte Nyvold)

**Finland (FI):**

Turku (Veli Kairisto)
Helsinki (NN)

**Norway (NO):**

Oslo University Hospital (Dag Andre Nymoen)

**Sweden (SE):**

Uppsala University Hospital (NN)
Lund University Hospital (Thoas Fioretos)
Karolinska Hospital, Stockholm (NN)
Umeå University Hospital (NN)
Linköping, University Hospital (NN)
PARTICIPATING IMMUNOLOGY AND STEM CELL LABORATORIES:

Dept of Immunology Uppsala (Angelica Loskog)

Hematology Research Unit, Helsinki University Hospital (Satu Mustjoki)

Section of Hematology, Inst of Internal Medicine, Bergen University (Bjørn Tore Gjertsen)

Dept of Clinical Genetics, Lund University (Thoas Fioretos, Johan Richter)

Dept of Clinical Immunology and Transfusion Medicine, Karolinska University Hospital Huddinge, Stockholm (Sarah Thunberg)
A SAFETY AND EFFICACY STUDY OF ADDING LOW DOSE PEGYLATED IFN-ALPHA 2B TO STANDARD DOSE DASATINIB IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA (NordCML007)

A stem cell subprotocol no 1:

Stem cell identification and quantification in predicting response to treatment with dasatinib±interferon in patients with newly diagnosed chronic myeloid leukemia.

Protocol Version Number: 2.0

Authors: S. Mustjoki, K. Porkka, L. Stenke, T. Fioretos and J. Richter
SYNOPSIS

Laboratory (stem cell) Protocol for NORDCML007

Title of Study: Stem cell identification and quantification in predicting response to treatment with dasatinib±interferon in patients with newly diagnosed chronic phase chronic myeloid leukemia (CP CML).

Estimated Number of Study Centers and Countries/Regions: Approximately 3-4 sites in 4 Nordic countries

Research Hypothesis: Leukemic stem cell (LSC) burden at diagnosis of CP CML and after 3 months of dasatinib therapy predicts long term molecular response to dasatinib±interferon therapy.

Objectives: To determine the proportion of LSCs in the stem cell populations in newly diagnosed CP CML patients at diagnosis and after 3 months of dasatinib treatment (100 mg QD) and correlate to subsequent treatment response to dasatinib±interferon.

Study Design: This is a substudy for the clinical protocol (NORDCML007) in newly diagnosed CP Ph+ CML subjects comparing dasatinib at a starting dose of 100 mg QD and dasatinib in combination with IFN-α. Bone marrow (BM) samples will be collected at diagnosis and 3 months after the start of dasatinib therapy. Proportion of leukemic stem cells will be determined by two methods: a. stem cell fractions from BM samples will be separated and the proportion of Ph+ cells will be analyzed. b. The frequency of IL1RAP-positive CD34+/CD38neg cells will be determined by FACS.

Duration of Study:

The study will be open for enrolment until the planned number of samples from approximately 35 subjects is collected

Number of Subjects: Approximately 35 subjects.

Study Population: Subjects 18 years or older with a newly diagnosed Ph+ CP CML, not previously treated with any systemic treatments for CML except for anagrelide or hydroxyurea. Subjects are treated according to the related clinical study protocol (NORDCML007 protocol).

Primary and Secondary Endpoints:

The primary endpoint is to determine whether proportion of LSCs in the stem cell population in newly diagnosed chronic CP CML patients at diagnosis correlates to subsequent response to dasatinib±interferon (MMR at 12 months). The secondary aims of the study are: i) the correlation of LSC burden at diagnosis with CMR rate at 18 months. ii) the correlation of LSC burden at 3 months with MMR rate at 12 months iii) the correlation of LSC burden at 3 months with CMR rate at 18 months and iv) the correlation LSC burden at diagnosis to hematological toxicity v) the comparison of the two methods to identify LSCs.
**Safety**

Bone marrow collection occurs according to normal routine procedures and no special safety aspects are foreseen.
<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE PAGE</td>
</tr>
<tr>
<td>SYNOPSIS</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
</tr>
<tr>
<td>1 INTRODUCTION AND STUDY RATIONALE</td>
</tr>
<tr>
<td>1.1 Research Hypothesis</td>
</tr>
<tr>
<td>1.2 Study Rationale</td>
</tr>
<tr>
<td>2 STUDY OBJECTIVES</td>
</tr>
<tr>
<td>2.1 Primary Objective</td>
</tr>
<tr>
<td>2.2 Secondary Objectives</td>
</tr>
<tr>
<td>3 ETHICAL CONSIDERATIONS</td>
</tr>
<tr>
<td>3.1 Good Clinical Practice</td>
</tr>
<tr>
<td>3.2 Institutional Review Board/Independent Ethics Committee</td>
</tr>
<tr>
<td>3.3 Informed Consent</td>
</tr>
<tr>
<td>4 INVESTIGATIONAL PLAN</td>
</tr>
<tr>
<td>4.1 Study Design and Duration</td>
</tr>
<tr>
<td>4.2 Study Population</td>
</tr>
<tr>
<td>5 STUDY ASSESSMENTS AND PROCEDURES</td>
</tr>
<tr>
<td>5.1 Flow chart/Time and Events Schedule</td>
</tr>
<tr>
<td>5.2 Study Materials</td>
</tr>
<tr>
<td>5.3 Procedures by Visit</td>
</tr>
<tr>
<td>6 STATISTICALS CONSIDERATIONS</td>
</tr>
<tr>
<td>6.1 Sample Size Determination</td>
</tr>
<tr>
<td>6.2 Populations for Analyses</td>
</tr>
<tr>
<td>6.3 Endpoint Definitions</td>
</tr>
</tbody>
</table>
6.4 Analyses 8
6.5 Interim analyses 9
7 LIST OF ABBREVIATIONS 9
8 REFERENCES 9
1 INTRODUCTION AND STUDY RATIONALE

1.1 Research Hypothesis

Leukemic stem cell (LSC) burden at diagnosis of CP CML and after 3 months of dasatinib therapy predicts long term molecular response to dasatinib±interferon therapy.

1.2 Study Rationale

Introduction to CML and drugs used in this protocol are described in the clinical NORDCML007 protocol. Following paragraphs are related to the stem cell substudy (substudy no 1).

Targeted tyrosine kinase inhibitor (TKI) imatinib efficiently induces rapid hematologic and cytogenetic remission in most CML patients. However, a small population of resistant primitive leukaemia stem cells remains even after years on therapy (Bhatia et al. 2003; Bocchia et al. 2007). Also in vitro experiments have shown that CML stem cells are resistant to TKIs (Graham et al. 2002; Copland et al. 2006). The clinical implication of stem cell resistance is a rapid leukaemia relapse in patients who discontinue imatinib. This residual population also serves as a reservoir for development of drug resistant clones, as it has been shown that most often drug resistance arises as a result of kinase domain mutations in the stem or progenitor cell compartment that affect imatinib binding.

The eradication or immunological control of the leukemia stem cells are prerequisites for cure. Potential strategies include breaking of the tumor immune tolerance or direct stem cell targeting with novel drugs, or a combination of both. In this setting, CML is an ideal model disease as the leukemic stem cells can readily be isolated from the bone marrow or blood, the disease is known to be immunogenic and most patients achieve a stable very low tumor burden.

Second generation TKIs have been shown to have a more profound effect on the stem cell compartment when compared to imatinib, but were still unable to kill the most primitive Ph+ CD34+CD38neg LSCs in vitro. In IM treated patients, the speed of reduction of BCR-ABL copies and clinical risk scores predict eventual outcome. For dasatinib patients these analyses have not been fully performed. In order to permanently eradicate CML, control of leukemic stem cells is essential. Whether 2nd generation TKIs are more effective in terms of LSC reduction or even complete eradication in vivo in patients is currently unknown. In addition, based on our own experience, the ratio of LSCs/normal hematopoietic stem cells (LSC/HSC ratio) at diagnosis and the kinetics of LSC disappearance may bear prognostic significance for response prediction and clinical outcome.

Before the TKI therapy era, interferon alpha (IFN-α) was the treatment of choice in CML. Only a small proportion of patients (10-20%) achieved a complete cytogenetic remission (CCyR), but these patients had a prolonged survival (Talpaz, Ann Intern Med 1991). Recent multicenter studies have shown that combination of IFN-α with the TKI imatinib improves the therapy outcome (Simonsson submitted 2011; Preudhomme NEJM 2011). Also studies evaluating the successful treatment discontinuation in CML have suggested that IFN-α therapy may improve the possibility to stop TKI therapy (Burchert J Clin Oncol
The mechanism of action of IFN-α therapy is incompletely understood; the drug exerts both direct cytostatic and immunomodulatory effects on leukemic cells. It can down-regulate the expression of the BCR-ABL1 gene, and activate several transcriptional factors that regulate cell proliferation, maturation, and apoptosis. IFN-α can also induce recognition and elimination of CML cells by the immune system. The most striking evidence of the immunomodulatory effects of IFN-α comes from studies which have shown that a significant proportion of IFN-α treated patients in prolonged CCyR were able to discontinue treatment without imminent disease relapse (Mahon, J Clin Oncol 2002). However, many of these patients still have detectable minimal residual disease. Recent studies have also suggested that IFN can promote the cycling of normal quiescent hematopoietic stem cells (Essers, Nature 2009). If similar mechanism of action occurs with dormant leukemic stem cells (LSCs), IFN-α treatment may induce their cycling and thereby expose LSCs to the effects of TKIs and chemotherapeutic agents.

Recently, we used gene-expression profiling to identify IL-1 receptor accessory protein (IL1RAP) as up-regulated in CML CD34+ cells and also in cord blood CD34+ cells as a consequence of retroviral BCR/ABL1 expression (Jarås et al, PNAS 2010). We further showed that IL1RAP expression distinguishes normal (Ph−) and leukemic (Ph+) cells within the more primitive CML CD34+CD38− cell compartment at diagnosis as CML CD34+CD38−IL1RAP+ cells were Ph+, whereas CML CD34+CD38−IL1RAP− cells were almost exclusively Ph−. By performing long-term culture-initiating cell assays on the two cell populations, we found that Ph+ and Ph− candidate CML stem cells could be prospectively separated. We thus identified IL1RAP as a unique cell surface biomarker distinguishing Ph+ from Ph− candidate CML stem cells and this has opened up a previously unexplored avenue for diagnostics and therapy of CML.

The number of CML patients analyzed for ILRAP-expression in the CD34+CD38neg population is still rather small and mainly restricted to diagnostic samples. Thus, the side-by-side analysis of LSC burden using the two separate methods described in this protocol, within the context of a clinical trial, will yield valuable information about LSC burden at diagnosis, during therapy with dasatinib and role of these parameters for response to therapy with dasatinib+interferon.

2 STUDY OBJECTIVES

2.1 Primary Objective

To determine the proportion of leukemic stem cells (LSC) in the stem cell populations in newly diagnosed chronic (CP) CML patients at diagnosis and correlate to subsequent treatment response to dasatinib±interferon (achievement of MMR at 12 months).
2.2 Secondary Objectives

- To compare the following study parameters:

  The proportion of LSCs in the stem cell populations in newly diagnosed chronic CML patients at diagnosis and achievement of non-detectable disease (CMR at 18 months) on treatment with dasatinib±interferon.

  The proportion of LSCs in the stem cell populations after 3 months of dasatinib treatment (100 mg QD) and subsequent molecular treatment response to dasatinib±interferon (MMR at 12 months and CMR 18 months).

  The proportion of LSCs in the stem cell populations at diagnosis and hematological toxicity on treatment with dasatinib±interferon.

  The two different methods to identify LSCs.

3 ETHICAL CONSIDERATIONS

3.1 Good Clinical Practice

This study will be conducted in accordance with Good Clinical Practice (GCP), as defined by the International Conference on Harmonisation (ICH) and in accordance with the ethical principles underlying European Union Directive 2001/20/EC and the United States Code of Federal Regulations, Title 21, Part 50 (21CFR50). The study will be conducted in compliance with the protocol. The protocol and any amendments and the subject informed consent has to receive Institutional Review Board/Independent Ethics Committee (IRB/IEC) approval/favorable opinion before initiation of the study. Study personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective task(s).

3.2 Institutional Review Board/Independent Ethics Committee

Before study initiation, the investigator must have written and dated approval/favourable opinion from the IRB/IEC for the protocol, consent form, subject recruitment materials/process (e.g., advertisements), and any other written information to be provided to subjects. The investigator or sponsor should also provide the IRB/IEC with a copy of the Investigator Brochure or product labeling, information to be provided to subjects and any updates. The investigator or sponsor should provide the IRB/IEC with reports, updates and other information (e.g., expedited safety reports, amendments, and administrative letters) according to regulatory requirements or institution procedures.
3.3 Informed Consent

Investigators must ensure that subjects are clearly and fully informed about the purpose, potential risks, and other critical issues regarding the studies in which they volunteer to participate. Freely given written informed consent must be obtained from every subject prior to study participation.

4 INVESTIGATIONAL PLAN

4.1 Study Design and Duration

4.1.1 Study Design

This study is a substudy for the clinical protocol (NORDCML007) in newly diagnosed CP Ph+ CML subjects comparing dasatinib at a starting dose of 100 mg QD and dasatinib in combination with IFN-α. Bone marrow samples will be collected at diagnosis and 3 months after the start of the therapy. Proportion of leukemic stem cells will be determined by two methods: a. Stem cell fractions from BM samples will be separated and the proportion of Ph+ cells will be analyzed. b. The frequency of IL1RAP-positive CD34+/CD38neg cells will be determined by FACS.

4.1.2 Study Duration

The study will be open for enrolment until the planned number of samples from approximately 35 subjects is collected

4.2 Study Population

Subjects 18 years or older with a newly diagnosed Ph+ CP CML, not previously treated with any systemic treatments for CML except for anagrelide or hydroxyurea. Subjects are treated according to the related clinical study protocol (NORDCML007 protocol). Inclusion and exclusion criteria are accordant with the clinical study protocol

5. STUDY ASSESSMENTS AND PROCEDURES

5.1 Flow Chart/Time and Events Schedule

Table 5.1 Flowchart
### 5.2 Study Materials

Bone marrow is done according to routine procedure. If the collection centre is far from the stem cell analysis centre, EDTA or heparin tubes will be used for collection/transport of the BM samples.

### 5.3 Procedures by Visit

Informed consent must be obtained prior to any study required procedures that would not have been performed as part of normal subject care.

Preferably 30 ml or more BM aspirate sample (EDTA or heparin anticoagulated) is obtained under local anaesthesia from study subjects. Samples are sent to qualified research (stem cell) laboratories (4 different centres) where the following procedures are performed.

1. Mononuclear cell fraction is separated from the BM aspirate sample by Ficoll centrifugation. Pre-separation of CD34-positive cells is done with para-magnetic beads. Pre-separated CD34+ cells are stained with CD34 and CD38 antibodies after which stem cell fractions are sorted with flow cytometry (FACSAria) (see gating procedure below). After sorting, cytospin slides are made from different stem cell fractions and additionally from un-separated whole BM. Proportion of Ph+ cells is determined with fluorescence in situ hybridization (FISH) method using BCR-ABL probe.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Pre-treatment</th>
<th>Month 3</th>
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</thead>
<tbody>
<tr>
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<td></td>
</tr>
<tr>
<td>Collection of Bone marrow for LSC 1 (30 ml)*</td>
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</tr>
<tr>
<td>Collection of Bone marrow for LSC 2 (10 ml)**</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

**Pre-separated CD34+ cells**

**Sorting of CD34+ cells**
2. Mononuclear cell fraction is separated from the BM aspirate sample by Ficoll centrifugation. MNCs are stained with CD34, CD38 and IL1RAP antibodies after which IL1RAP expression will be determined on the CD34+/CD38neg and CD34+/CD38+ populations.

6 STATISTICAL CONSIDERATIONS

6.1 Sample Size Determination

An appropriate estimation of the sample size needed is not possible to be made, as this is an explorative study. It is however estimated that with the analysis of 35 patients trends could be observed which could be confirmed in bigger sample size setting.

6.2 Populations for Analyses

The following data sets will be used in this study:

- All enrolled subjects: All subjects who signed an informed consent form and were registered.
- All evaluable subjects: All subjects who have at least one adequate and good-quality on-study sample available for the analysis.

All evaluable subjects will be used in the analysis of baseline characteristics and later follow-up time points.

6.3 Endpoint Definitions

The primary endpoint to define whether proportion of LSCs in the stem cell population in newly diagnosed chronic CP CML patients at diagnosis correlates to subsequent molecular response to dasatinib±interferon (MMR at 12 months).

Secondary endpoints include the
i) correlation of LSC burden at diagnosis with CMR rate at 18 months.
ii) correlation of LSC burden at 3 months with MMR rate at 12 months.
iii) correlation of LSC burden at 3 months with CMR rate at 18 months.
iv) correlation LSC burden at diagnosis to hematological toxicity.
v) comparison of the two methods to identify LSCs.

6.4 Analyses
6.4.1 Demographics and Baseline Characteristics

Demographic and baseline characteristics will be tabulated by arm using descriptive statistics.

6.5 Interim Analyses

Interim analysis of the material will be done when 20 subjects are enrolled to the study.

7 LIST OF ABBREVIATIONS

CML Chronic myeloid leukemia
CP Chronic phase
CTCAE Common Terminology Criteria for Adverse Events
GCP Good Clinical Practice
IFN Interferon
IL1RAP IL-1 receptor accessory protein
IRB Institutional review board
IEC Independent ethics committee
LSC Leukemic stem cell
PB Peripheral blood
Ph+ Philadelphia chromosome positive
QD once daily
TKI tyrosine kinase inhibitor

8 REFERENCES


Bocchia, M., M. Ippoliti, et al. (2007). "CD34+/Ph+ cells are still detectable in chronic myeloid leukemia patients with sustained and prolonged complete cytogenetic remission during treatment with imatinib mesylate." Leukemia.


A SAFETY AND EFFICACY STUDY OF ADDING LOW DOSE PEGYLATED IFN-ALPHA 2B TO STANDARD DOSE DASATINIB IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA (NordCML007)

An immunology subprotocol no 2:

Characterization of predictive factors and immunological monitoring of effects of dasatinib and IFN-α in chronic myeloid leukemia patients in chronic phase

Protocol Version Number: 2.0

Authors: S.Mustjoki, A. Loskog, K. Porkka
SYNOPSIS

Laboratory (immunology) Protocol for NORDCML007

Title of Study: Characterization of predictive factors and immunological monitoring of effects of dasatinib and IFN-α in chronic myeloid leukemia patients in chronic phase. A substudy no 2 for NordCML007 protocol.

Estimated Number of Study Centers and Countries/Regions: Approximately 2-3 sites in 4 Nordic countries

Research Hypothesis: Treatment with dasatinib and IFN-α combination therapy results in more profound reduction in long-term disease burden and induces immunological changes, which could facilitate superior treatment responses compared to tyrosine kinase inhibitor monotherapy.

Objectives: To define whether dasatinib alone and/or dasatinib and IFN-α combination therapy induces numerical or functional immunological changes as assessed by flow cytometry and functional assays of peripheral blood samples at 3 and 12 month time points after the therapy start.

Study Design: This study is a substudy for the clinical protocol (NORDCML007) in newly diagnosed CP Ph+ CML subjects studying the effect of dasatinib 100 mg QD in combination with IFN-α. Peripheral blood samples will be collected at diagnosis and after 3 and 12 months after the start of the therapy. 3 months time-point detects changes induced by dasatinib before IFN-α administration and 12 month time point evaluates the effects of combination regimen. The immunophenotype, clonality and the function of cells will be analyzed.

Duration of Study:

The study will be open for enrolment until the planned number of samples from approximately 35 subjects is collected. Blood samples will be collected for up to 12 months, and in case of immunological adverse effects/symptoms, samples can be obtained also at later time points.

Number of Subjects: Approximately 35 subjects, only one treatment arm (dasatinib and IFN-α combination regimen)

Study Population: Subjects 18 years or older with a newly diagnosed Ph+ CP CML, not previously treated with any systemic treatments for CML except for anagrelide or hydroxyurea. Subjects are treated according to the related clinical study protocol (NORDCML007 protocol).

Primary and Secondary Endpoints:

The primary endpoint is to define whether dasatinib alone and/or dasatinib and IFN-α combination therapy induces numerical or functional immunological changes as assessed by flow cytometry and functional assays of peripheral blood samples. The secondary aims of the study are: to correlate the immunological effects of therapy with achievement of complete molecular response at 12 months and (b) with autoimmune like side-effects. (c) To find biological markers
which could predict which patients are able to discontinue the therapy without disease relapse.

**Safety**

Blood sample collection occurs according to normal routine procedures and no special safety aspects are foreseen.
TABLE OF CONTENTS

TITLE PAGE 1
SYNOPSIS 2
TABLE OF CONTENTS 3
1 INTRODUCTION AND STUDY RATIONALE 4
1.1 Research Hypothesis 4
1.2 Study Rationale 4
2 STUDY OBJECTIVES 5
2.1 Primary Objective 5
2.2 Secondary Objectives 5
3 ETHICAL CONSIDERATIONS 5
3.1 Good Clinical Practice 5
3.2 Institutional Review Board/Independent Ethics Committee 5
3.3 Informed Consent 6
4 INVESTIGATIONAL PLAN 6
4.1 Study Design and Duration 6
4.2 Study Population 6
5 STUDY ASSESSMENTS AND PROCEDURES 7
5.1 Flow chart/Time and Events Schedule 7
5.2 Study Materials 7
5.3 Procedures by Visit 7
6 STATISTICS CONSIDERATIONS 8
6.1 Sample Size Determination 8
6.2 Populations for Analyses 8
6.3 Endpoint Definitions 8
6.4 Analyses 8
6.5 Interim analyses 9
7 LIST OF ABBREVIATIONS 9
8 REFERENCES 9
1 INTRODUCTION AND STUDY RATIONALE

1.1 Research Hypothesis
Treatment with dasatinib and IFN-α combination therapy results in more profound reduction in long-term disease burden and induces immunological changes, which could facilitate superior treatment responses than dasatinib monotherapy in newly diagnosed chronic phase CML patients.

1.2 Study Rationale
Introduction for CML and drugs used in the clinical protocol are described in the related clinical NORDCML007 protocol. Following paragraphs are related to the immunological substudy (substudy no 2).

Molecularly targeted therapy by tyrosine kinase inhibitors (TKIs) has revolutionized the treatment of chronic myeloid leukemia during last 10 years. As the drug therapy is not curative, life-long, significant long-term off-target toxicities on normal cells and tissues may emerge. However, some of these effects may turn out to be clinically beneficial.

The eradication or immunological control of the leukemia stem cells are prerequisites for cure. Potential strategies include breaking of the tumor immune tolerance or direct stem cell targeting with novel drugs, or a combination of both. In this setting, CML is an ideal model disease as the leukemic stem cells can readily be isolated from the bone marrow or blood, the disease is known to be immunogenic and most patients achieve a stable very low tumor burden.

The kinase inhibition profile of second generation TKIs like dasatinib is broad. In addition to BCR-ABL dasatinib also inhibits kinases, which have important function in immune cells (like src-kinases). In vitro, dasatinib, but also imatinib and nilotinib have shown to have immunosuppressive effects on T- and NK-cells. In patients, however, the effects can be different. In our earlier studies we noticed that in a proportion of patients, dasatinib reverted the immune tolerance and induced a massive clonal expansion of cytotoxic LGL (large granular lymphocyte)-cells in peripheral blood (Mustjoki et al, Leukemia 2009). The expansion of immune effector cells was linked to autoimmune reactivity ("LGL-versus-host"), such as colitis or pneumonitis, as accumulation of clonal T/NK-cells was also observed in the target organs. Furthermore, several patients with advanced, poor-prognosis leukemia achieved long-lasting complete responses to dasatinib, thus strongly implying a direct antitumor effect of the cytotoxic cells ("LGL-versus-leukemia"). The immunomodulatory effects of dasatinib are distinct from other TKIs and this could be beneficial in the future when assessing which patients are able to discontinue the treatment with relapse.

Before the TKI therapy era, interferon alpha (IFN-α) was the treatment of choice in CML. Only a small proportion of patients (10-20%) achieved a complete cytogenetic remission (CCyR), but these patients had a prolonged survival
Recent multicenter studies have shown that combination of IFN-α with the TKI imatinib improves the therapy outcome (Simonsson submitted 2011; Preudhomme NEJM 2011). Also studies evaluating the successful treatment discontinuation in CML have suggested that IFN-α therapy may improve the possibility to stop TKI therapy (Burchert J Clin Oncol 2010). The mechanism of action of IFN-α therapy is incompletely understood; the drug exerts both direct cytostatic and immunomodulatory effects on leukemic cells. It can down-regulate the expression of the BCR-ABL1 gene, and activate several transcriptional factors that regulate cell proliferation, maturation, and apoptosis. IFN-α can also induce recognition and elimination of CML cells by the immune system. The most striking evidence of the immunomodulatory effects of IFN-α comes from studies which have shown that a significant proportion of IFN-α treated patients in prolonged CCyR were able to discontinue treatment without imminent disease relapse (Mahon, J Clin Oncol 2002). However, many of these patients still have detectable minimal residual disease.

We have also shown previously that IFN monotherapy induces distinct changes in the immunoprofile of CML patients, which may contribute to prolonged therapy responses in this unique group of patients. IFN-treated CML patients in remission had increased numbers of NK-cells and clonal γδ+ T-cells and a unique plasma cytokine profile (Kreutzman, ASH2010).

It would be important in clinical setting to understand the mechanisms of drug-induced cure and to assess which factors are important in the maintenance of residual tumor cell dormancy.

2 STUDY OBJECTIVES

2.1 Primary Objective

To define whether dasatinib alone and/or dasatinib and IFN-α combination therapy induces numerical or functional immunological changes as assessed by flow cytometry and functional assays of peripheral blood samples at 3 and 12 month time points after the therapy start.

2.2 Secondary Objectives

• To compare the following study parameters overall:

  The immunological effects of therapy with achievement of cytogenetic and molecular genetic responses at 3, 6, 12 and 24 months

  The immunological effects of therapy with autoimmune like side-effects

• To find biological markers which could predict which patients are able to discontinue the therapy without disease relapse
3 ETHICAL CONSIDERATIONS

3.1 Good Clinical Practice

This study will be conducted in accordance with Good Clinical Practice (GCP), as defined by the International Conference on Harmonisation (ICH) and in accordance with the ethical principles underlying European Union Directive 2001/20/EC and the United States Code of Federal Regulations, Title 21, Part 50 (21CFR50). The study will be conducted in compliance with the protocol. The protocol and any amendments and the subject informed consent has to receive Institutional Review Board/Independent Ethics Committee (IRB/IEC) approval/favorable opinion before initiation of the study. Study personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective task(s).

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4 INVESTIGATIONAL PLAN

4.1 Study Design and Duration

4.1.1 Study Design

This study is a substudy for the clinical protocol (NORDCML007) in newly diagnosed CP Ph+ CML subjects studying the effect of dasatinib 100 mg QD in combination with IFN-α. Peripheral blood samples will be collected at diagnosis and after 3 and 12 months after the start of the therapy. 3 months time-point detects changes induced by dasatinib before IFN-α administration and 12 months time-point evaluates the effects of combination regimen. The immunophenotype, clonality and the function of cells will be analyzed.

4.1.2 Study Duration
The study will be open for enrolment until the planned number of samples from approximately 35 subjects is collected. Blood samples will be collected for up to 12 months, and in case of immunological adverse effects/symptoms, samples can be obtained also at later time points.

4.2 Study Population

Subjects 18 years or older with a newly diagnosed Ph+ CP CML, not previously treated with any systemic treatments for CML except for anagrelide or hydroxyurea. Subjects are treated according to the related clinical study protocol (NORDCML007 protocol). Inclusion and exclusion criteria are accordant with the clinical study protocol.

5. STUDY ASSESSMENTS AND PROCEDURES

5.1 Flow Chart/Time and Events Schedule

<table>
<thead>
<tr>
<th>Immunology/stem cell substudies</th>
<th>On treatment visits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procedure: See Appendix 3 and 5 for details</td>
<td>Pre-treatment</td>
</tr>
<tr>
<td>Lymphocyte studies: Collection of PB sample (30ml heparinized blood) before drug intake</td>
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</tr>
<tr>
<td>Dasatinib plasma concentration and differential count (One 5ml EDTA tube) before and 1 h after dasatinib</td>
<td>X</td>
</tr>
<tr>
<td>Phosphoflow: (5 ml citrate tube) before and 1 h after dasatinib</td>
<td>X</td>
</tr>
</tbody>
</table>

1 Samples for phosphoflow must be taken before and 1h after intake of dasatinib. Store locally in -80°C freezer and ship in larger batches (on dry ice). See Appendix 5 for instruction in detail. If there are considerable problems for the patient (i.e long travel) to come to clinic on day 1 for phosphoflow, a baseline sample at the pretreatment visit is also very valuable

5.2 Study Materials

Peripheral blood collection is done according to routine procedure. Blood will be collected to heparinized tubes and sent immediately to analytical centres. Dasatinib plasma concentration measurements will be done from EDTA plasma and for that 5 ml of blood will be collected in EDTA anticoagulant tubes.

5.3 Procedures by Visit

Informed consent must be obtained prior to any study required procedures that would not have been performed as part of normal subject care.

Flow cytometry panel:
Tube 1: Effector memory/naïve/central memory
Tube 2: TCR /b vs g/d cells
Tube 3: Lymphocyte subclasses: T, B, NK, NKT cells
Tube 4: Activation of T-cells
Tube 5: Myeloid suppressor cells
Tube 6: Regulatory T-cells
Tube 7: Dendritic cells

**Functional assays:**

The functional assays will be done from frozen stored cells. The cytotoxicity of NK-cells will be analyzed by using normal cytotoxicity assay with K562 as target cells. T-cell activation will be determined with IFN-γ production from CD4+ and CD8+ cells after OKT-3 and CD28 antibody stimulation. Granzyme B staining will be used for evaluating the cytotoxic potential of T-cells. The functional assays will

<table>
<thead>
<tr>
<th>Tube</th>
<th>FITC</th>
<th>PE</th>
<th>PerCP</th>
<th>PE-Cy7</th>
<th>APC</th>
<th>APC-Cy7</th>
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<tr>
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<td>CD45RO</td>
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<td>BDCA1</td>
<td>CD14</td>
<td>BDCA3</td>
<td>CD19</td>
<td></td>
</tr>
</tbody>
</table>
be performed both from pre-drug samples.

**Clonality assays:**

The cell clonality will be determined by PCR based assay detecting TCR γδ rearrangements. Positive products will be confirmed by sequencing. The clonality assay will be performed from post-drug sample.

**Plasma cytokine array:**

Plasma cytokines will be determined by using Luminex array detecting 25 different cytokines.

6 STATISTICAL CONSIDERATIONS

6.1 Sample Size Determination

An appropriate estimation of the sample size needed is not possible to be made as this is an explorative study. It is however estimated that with the analysis of 20 patients from both groups trends could be observed which could be confirmed in bigger sample size setting.

6.2 Populations for Analyses

The following data sets will be used in this study:

- All enrolled subjects: All subjects who signed an informed consent form and were registered.
- All evaluable subjects: All subjects who have at least one adequate and good-quality on-study sample available for the analysis.

All evaluable subjects will be used in the analysis of baseline characteristics and later follow-up time points.

6.3 Endpoint Definitions

The primary endpoint to define whether dasatinib alone and/or dasatinib and IFN-α combination therapy induces numerical or functional immunological changes as assessed by flow cytometry and functional assays of peripheral blood samples.

Secondary endpoints include the correlation of the immunological effects of therapy with the achievement of complete molecular response at 12 months and (b) with autoimmune like side-effects. (c) The detection of biological markers which could predict which patients are able to discontinue the therapy without disease relapse.

6.4 Analyses
6.4.1 Demographics and Baseline Characteristics

Demographic and baseline characteristics will be tabulated by arm using descriptive statistics.

6.5 Interim Analyses

Interim analysis of the material will be done when 20 subjects are enrolled to the study.

7 LIST OF ABBREVIATIONS

CML Chronic myeloid leukemia
CP Chronic phase
CTCAE Common Terminology Criteria for Adverse Events
GCP Good Clinical Practice
IFN Interferon
IRB Institutional review board
IEC Independent ethics committee
PB Peripheral blood
Ph+ Philadelphia chromosome positive
QD once daily
TKI tyrosine kinase inhibitor

8 REFERENCES


GCP
The investigator agrees, by signing the protocol, to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice, as of CPMP/ICH/135/95 and to the Declaration of Helsinki (with latest amendment WMA General Assembly 2002).

All source documents and the complete study documentation will be retained for at least 15 years at the clinical centre. The sponsor will communicate when the documentation can be destroyed.

INSTITUTIONAL REVIEW BOARD ETHICS COMMITTEE
The final approved protocol and the informed consent will be reviewed and approved by the local EC. The approval of the study will be made in writing to the investigator and a copy of this decision will be provided to the Sponsor before the commencement of the study. This approval form should report title and code of the study, a list of the examined documentation, list of members of the meeting session, date and outcome of meeting. Any amendment of the protocol and/or consent form has to be approved by the EC.

INFORMED CONSENT
Prior to entering the study, the patient will be informed of the nature of the study drugs and study procedures and will give in writing his/her consent to participate to the study. The consent statement will then be read and signed by the patient and the investigator.

PROTOCOL MODIFICATIONS
Any modification which may impact on the conduct of the study or may affect patient safety, including changes of study objectives, study design, patient population, study procedures, or significant administrative aspects will require a formal amendment to the protocol. The Sponsor, the investigators, and the Ethics Committee prior to implementation will agree upon such amendment.

Administrative changes of the protocol are minor corrections, which have no impact on the conduction of the study. These administrative changes will be agreed upon by the Sponsor and the principal investigator. The Ethics Committee should be notified of these administrative changes.

MONITORING
Monitoring will be performed by research nurses, trained in clinical trial monitoring, on charge of the Danish/Finnish/Norwegian/Swedish CML Study
Groups. Local investigators must co-operate with monitors and consent direct access to source data and documents.

Each study centre should be monitored at least once during the study period.

At the visit the monitor shall control:

* Patient ID and informed consent
* That the consent form is dated and signed correctly
* That inclusion and exclusion criteria are correct.
* That analysis for primary endpoint is performed in a correct way.
* That SAEs have been handled correct according to study protocol
* That relevant study documentation is available in the patient records.
* Investigators file

The monitor should write a monitoring report after each visit. This should contain a relevant description of what has been controlled. Discrepancies, defects and falsifications found and prospects for corrections of these shall immediately be documented and presented to study responsible personnel in a special report form.

The monitor should in advance have an agreement with responsible head of department to guarantee access to the study data.

The monitors visit should be documented in a monitoring visit log signed and dated by monitor and local investigator or study nurse. This monitoring visit log should be added to the study file.

**Drug accountability**

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and assure appropriate supply, handling, storage, distribution, and usage of these materials in accordance with the protocol and any applicable laws and regulations. Clinical supplies may not be used for any purpose other than that stated in the protocol.

**Treatment monitoring committee (TMC)**

A TMC will be appointed. It is the responsibility of the TMC,

* to monitor SAE in real time
* to authorise to resume the treatment if it was discontinued for a SAE that could not be attribute to study drugs.
* to activate the study co-ordinators for any relevant action or communication in the interest of the patients and of the study.
September 9th, 2004
Appendix 5 Phosphoproteomics

1. Tyrosine Kinase Inhibitor (TKI) pharmacodynamics (PD) analysis

1.1. Timing of assessment

See below for timing and instruction of peripheral blood sampling for phenotyping and functional testing.

Sampling

Day 1: Sampling just PRIOR the first drug intake and 1 hour AFTER the first drug intake (=2 samplings).

Start of month 3: Sampling just PRIOR the first drug intake and 1 hour AFTER the first drug intake (=2 samplings).

Citrate blood sample for PD analysis: 5 ml

1.2. Blood sampling for TKI PD analysis

a. Materials and instrumentation:

- +37°C water bath
- Sample centrifuge (equipped with a tube bucket flexible rotor, +4°C)
- BD Phosflow Lyse/Fix Buffer (5x concentration) (BD Biosciences #558049)
- 0.9% NaCl, +4°C
- ddH2O
- 50 ml tube (Sarstedt #62.547 or equivalent)
- Cryo tubes 2 mL, tapered, polypropylene screw-cap tube (Sarstedt #72.693 or equivalent without a skirted base)
- -80°C freezer
b. **Collection, preparation and storage**

1. Prepare two 50 ml tubes with lyse/fix buffer.
   - For each tube add 9 ml BD Phosflow Lyse/Fix Buffer (5X concentrate) + 36 ml ddH2O and pre-warm to +37°C in water bath

2. Collect minimum 5 ml human blood in the presence of Citrate

3. Transfer 2.5 ml blood to each 50 ml tube with the pre-warmed (37°C) BD lyse/fix buffer (1X) (minimum 20X volumes of buffer)

4. Mix well by inverting the tubes 8 to 10 times

5. Incubate the tubes in a +37°C water bath for 10 minutes

6. Pellet the cells by centrifugation 500×g for 8 min, +4°C

7. Aspirate the supernatant and vortex the cell pellet

8. Wash the cells once with 30 mL 0.9% NaCl +4°C, and pellet by centrifugation 500×g for 8 min (+4°C) and remove the supernatant

9. Add 2 ml of 0.9% NaCl +4°C, make 2 aliquots of 1 ml in cryo tubes

10. Store directly at -80°C until shipping

**1.3. Blood sample shipment as frozen sample**

All patient samples should be shipped together after finalized inclusion of patients and all samples are collected. Contact central phospho-flow facility before shipping. Contact persons: Siv Lise Bedringaas (siv.bedringaas@med.uib.no, phone: +47 55 97 30 59), and Bjørn T. Gjertsen, PI (bjorn.gjertsen@med.uib.no, phone: +47 41 56 62 48).

Shipment on day from Monday – Wednesday. Please label tubes and containers clearly. Define type of sample. Please fill out and add a complete the transport document. Shipping temperature: dry ice (ship in 10 kg dry ice).
1.4. Sample transportation form and tube labeling

The sample transportation form is shown in *Sub Study Shipping Form*. This should be completed by the site prior to shipment.

Please complete all the required information with a black ball point pen. This information is required for patient identification and results reporting.

Any errors or missing details with regard to patient information will cause a delay in the reporting of the results until information is verified!

Send the original of the completed transportation form by fax to the Laboratory, keep a copy archived with all other patients related documents.

Complete the labels with appropriate identifiers and attach it to each tube sent to the Laboratory.

1.5. Collection of analysis results

Results will be collected to the web based database in analysis laboratory.

1.6. Storage of samples

All residual samples will be stored by the reference laboratory at least until the completion of the Clinical Study Report (anticipated mid 2014). Beyond this date
samples should be destroyed unless local or national guidelines indicate these should be retained for a further period.
Samples and sampling time-points:

- 5 ml of PB samples will be taken:
  - at day 1 **prior** to first drug intake (t=1)
  - at day 1, 1 hour **after** first drug intake (t=2)
  - at day 90 **prior** to first drug intake (t=3)
  - at day 90, 1 hour **after** first drug intake (t=4)

Materials:

- Vacutainer with sodium (natrium) citrate (NC), min. 4,5 ml (example BD vacutainer 9NC #367704)
- BD Phosflow Lyse/Fix Buffer (5x concentration)
- 0.9% NaCl , 4°C
- ddH₂O
- 50 ml tubes (polypropylene, Sarstedt #62.547, Falcon or equivalent)
- Cryo tubes (1.8 ml) (polypropylene, Sarstedt #72.379002 or equivalent). We suggest 2 tubes labelled with the same number. Label tubes with patient study number and number 1, 2, 3, and 4 indicating time of sample.

Sampling procedure:

- Prepare BD Phosflow Lyse/Fix Buffer:
  Two tubes (50 mL) containing 9 ml BD Phosflow Lyse/Fix Buffer + 36 ml deionized distilled H₂O and heat in water bath (37°C).
- Collect a minimum of 4,5 ml PB in the presence of sodium citrate.
- Transfer 2.5 ml of PB to each 50 mL tube with pre-heated (37°C) lyse/fix buffer and mix by inverting the tubes 10 times.
- Incubate the cells at 37°C for 10 min in water bath (37°C).
- Pellet the cells by centrifugation at 500xg for 8 min (temperature 4°C).
- Aspirate the supernatant and gently vortex the cell pellet.
- Wash the cells once with 30 mL 0.9% NaCl (4°C) and pellet (500xg 4°C for 8 min).
- Aspirate the supernatant and gently vortex the cell pellet.
- Add 2 ml (1 ml to each 50 ml tube) of 0.9% NaCl 4°C, mix and transfer into the cryo tubes.
- Store directly at -80°C until shipping on dry ice.

Shipment:
PHOSPHOFLOW SUB STUDY SHIPPING FORM

SAMPLING CONDITIONS/TRANSPORT DOCUMENT

PHARMACODYNAMICS

Sampling conditions

- Concerning the exact sampling procedure and local storing conditions see "PD-SOP".
- Keep the samples at -80 until all samples are included.
- Days of shipment: **Monday – Wednesday**.
- Please fill out the complete form for each individual patient and sampling days.
- Shipping temperature: Dry ice (10 kg).
- Shipment by World Courier (will be provided by _______ including all shipment document).
- In the case of questions please contact: Siv Lise Bedringaas, siv.bedringaas@med.uib.no, Phone: +47 55 97 30 59 or Bjørn T. Gjertsen, PI (bjorn.gjertsen@med.uib.no, Phone: +47 41 56 62 48).

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Start date: . . . . / . . . . ./ . . . . . Stop date: . . . . / . . . . ./ . . . . .
Required time points 5 ml PB (sodium citrate) is needed:

- Day 1; before intake of the first capsule (t = baseline = 1)
- Day 1; 1 hour after intake of the first capsule (t = 2)
- Day 90; before intake of the first capsule (t = 3)
- Day 90; 1 hour after intake of the first capsule (t = 4)

Collection date: . . . . / . . . . . / . . . . . collection time: . . . . and . . . . .